



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/31, C07K 14/35, C12N 15/62, C07K 19/00, 16/12, A61K 39/04, 48/00, G01N 33/68	A2	(11) International Publication Number: WO 99/32634 (43) International Publication Date: 1 July 1999 (01.07.99)																		
(21) International Application Number: PCT/NZ98/00189 (22) International Filing Date: 23 December 1998 (23.12.98) (30) Priority Data: <table border="0"><tr><td>08/997,362</td><td>23 December 1997 (23.12.97)</td><td>US</td></tr><tr><td>08/997,080</td><td>23 December 1997 (23.12.97)</td><td>US</td></tr><tr><td>08/996,624</td><td>23 December 1997 (23.12.97)</td><td>US</td></tr><tr><td>09/095,855</td><td>11 June 1998 (11.06.98)</td><td>US</td></tr><tr><td>09/156,181</td><td>17 September 1998 (17.09.98)</td><td>US</td></tr><tr><td>09/205,426</td><td>4 December 1998 (04.12.98)</td><td>US</td></tr></table> (71) Applicant (for all designated States except US): GENESIS RE- SEARCH & DEVELOPMENT CORPORATION LIMITED [NZ/NZ]; 1 Fox Street, Parnell, Auckland (NZ). (72) Inventors; and (75) Inventors/Applicants (for US only): TAN, Paul [NZ/NZ]; 26B Alberon Street, Parnell, Auckland (NZ). WATSON, James [NZ/NZ]; 769 Riddell Road, Auckland (NZ). VISSER, Elizabeth, S. [ZA/NZ]; 3 Lynbrooke Avenue, Blockhouse Bay, Auckland (NZ). SKINNER, Margot, A. [NZ/NZ]; 113 West End Road, Westmere, Auckland (NZ). PRESTIDGE, Ross, L. [NZ/NZ]; 20 Hepburn Street, Freemans Bay, Auckland (NZ).		08/997,362	23 December 1997 (23.12.97)	US	08/997,080	23 December 1997 (23.12.97)	US	08/996,624	23 December 1997 (23.12.97)	US	09/095,855	11 June 1998 (11.06.98)	US	09/156,181	17 September 1998 (17.09.98)	US	09/205,426	4 December 1998 (04.12.98)	US	(74) Agents: BENNETT, Michael, Roy et al.; Russell McVeagh West-Walker, The Todd Building, Level 5, 171-177 Lambton Quay, Wellington 6001 (NZ). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>
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(54) Title: COMPOSITIONS DERIVED FROM <i>MYCOBACTERIUM VACCAE</i> AND METHODS FOR THEIR USE (57) Abstract The present invention provides compositions which are present in or may be derived from <i>Mycobacterium vaccae</i> , together with methods for their use in the treatment, prevention and detection of disorders including infectious diseases, immune disorders and cancer. Methods for enhancing the immune response to an antigen including administration of <i>M. vaccae</i> culture filtrate, delipidated <i>M. vaccae</i> cells, delipidated and deglycolipidated <i>M. vaccae</i> cells depleted of mycolic acids, and delipidated and deglycolipidated <i>M. vaccae</i> cells depleted of mycolic acids and arabinogalactan are also provided.																				

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COMPOSITIONS DERIVED FROM MYCOBACTERIUM VACCAE AND METHODS FOR THEIR USE

Technical Field

The present invention relates generally to compositions which are present in or may be derived from *Mycobacterium vaccae* and their use in the treatment, prevention and detection of disorders including infectious diseases, immune disorders and cancer. In particular, the invention is related to compounds and methods for the treatment of diseases of the respiratory system, such as mycobacterial infections, asthma, sarcoidosis and lung cancers, and disorders of the skin, such as psoriasis, atopic dermatitis, allergic contact dermatitis, alopecia areata, and the skin cancers basal cell carcinoma, squamous cell carcinoma and melanoma. The invention is further related to compounds that function as non-specific immune response amplifiers, and the use of such non-specific immune response amplifiers as adjuvants in vaccination or immunotherapy against infectious disease, and in certain treatments for immune disorders and cancer.

Background of the Invention

Tuberculosis is a chronic, infectious disease, that is caused by infection with *Mycobacterium tuberculosis* (*M. tuberculosis*). It is a major disease in developing countries, as well as an increasing problem in developed areas of the world, with about 8 million new cases and 3 million deaths each year. Although the infection may be asymptomatic for a considerable period of time, the disease is most commonly manifested as a chronic inflammation of the lungs, resulting in fever and respiratory symptoms. If left untreated, significant morbidity and death may result.

Although tuberculosis can generally be controlled using extended antibiotic therapy, such treatment is not sufficient to prevent the spread of the disease. Infected individuals may be asymptomatic, but contagious, for some time. In addition, although compliance with the

treatment regimen is critical, patient behaviour is difficult to monitor. Some patients do not complete the course of treatment, which can lead to ineffective treatment and the development of drug resistant mycobacteria.

Inhibiting the spread of tuberculosis requires effective vaccination and accurate, early diagnosis of the disease. Currently, vaccination by subcutaneous or intradermal injection with live bacteria is the most efficient method for inducing protective immunity. The most common mycobacterium employed for this purpose is *Bacillus Calmette-Guerin* (BCG), an avirulent strain of *Mycobacterium bovis* (*M. bovis*). However, the safety and efficacy of BCG is a source of controversy and some countries, such as the United States, do not vaccinate the general public. Diagnosis of *M. tuberculosis* infection is commonly achieved using a skin test, which involves intradermal exposure to tuberculin PPD (protein-purified derivative). Antigen-specific T cell responses result in measurable induration at the injection site by 48-72 hours after injection, thereby indicating exposure to mycobacterial antigens. Sensitivity and specificity have, however, been a problem with this test, and individuals vaccinated with BCG cannot be distinguished from infected individuals.

A less well-known mycobacterium that has been used for immunotherapy for tuberculosis and also leprosy, by subcutaneous or intradermal injection, is *Mycobacterium vaccae* (*M. vaccae*), which is non-pathogenic in humans. However, there is less information on the efficacy of *M. vaccae* compared with BCG, and it has not been used widely to vaccinate the general public. *M. bovis* BCG and *M. vaccae* are believed to contain antigenic compounds that are recognised by the immune system of individuals exposed to infection with *M. tuberculosis*.

Several patents and other publications disclose treatment of various conditions by administering mycobacteria, including *M. vaccae*, or certain mycobacterial fractions. U.S. Patent 4,716,038 discloses diagnosis of, vaccination against and treatment of autoimmune diseases of various types, including arthritic diseases, by administering mycobacteria, including *M. vaccae*. U.S. Patent 4,724,144 discloses an immunotherapeutic agent comprising antigenic material derived from *M. vaccae* for treatment of mycobacterial diseases, especially tuberculosis and leprosy, and as an adjuvant to chemotherapy.

International Patent Publication WO 91/01751 discloses the use of antigenic and/or immunoregulatory material from *M. vaccae* as an immunoprophylactic to delay and/or prevent the onset of AIDS. International Patent Publication WO 94/06466 discloses the use of antigenic and/or immunoregulatory material derived from *M. vaccae* for therapy of HIV infection, with or without AIDS and with or without associated tuberculosis.

U.S. Patent 5,599,545 discloses the use of mycobacteria, especially whole, inactivated *M. vaccae*, as an adjuvant for administration with antigens which are not endogenous to *M. vaccae*. This publication theorises that the beneficial effect as an adjuvant may be due to heat shock protein 65 (hsp 65). International Patent Publication WO 92/08484 discloses the use of antigenic and/or immunoregulatory material derived from *M. vaccae* for the treatment of uveitis. International Patent Publication WO 93/16727 discloses the use of antigenic and/or immunoregulatory material derived from *M. vaccae* for the treatment of mental diseases associated with an autoimmune reaction initiated by an infection. International Patent Publication WO 95/26742 discloses the use of antigenic and/or immunoregulatory material derived from *M. vaccae* for delaying or preventing the growth or spread of tumors. International Patent Publication WO 91/02542 discloses the use of autoclaved *M. vaccae* in the treatment of chronic inflammatory disorders in which a patient demonstrates an abnormally high release of IL-6 and/or TNF or in which the patient's IgG shows an abnormally high proportion of agalactosyl IgG. Among the disorders mentioned in this publication are psoriasis, rheumatoid arthritis, mycobacterial disease, Crohn's disease, primary biliary cirrhosis, sarcoidosis, ulcerative colitis, systemic lupus erythematosus, multiple sclerosis, Guillain-Barre syndrome, primary diabetes mellitus, and some aspects of graft rejection.

M. vaccae is apparently unique among known mycobacterial species in that heat-killed preparations retain vaccine and immunotherapeutic properties. For example, *M. tuberculosis* BCG vaccines, used for vaccination against tuberculosis, employ live strains. Heat-killed *M. bovis* BCG and *M. tuberculosis* have no protective properties when employed in vaccines. A number of compounds have been isolated from a range of mycobacterial

species which have adjuvant properties. The effect of such adjuvants is essentially to stimulate a particular immune response mechanism against an antigen from another species.

There are two general classes of compounds which have been isolated from mycobacterial species that exhibit adjuvant properties. The first are water soluble wax D fractions (R.G. White, I. Bernstock, R.G.S. Johns and E. Lederer, Immunology, 1:54, 1958; US Patent 4,036,953). The second are muramyl dipeptide-based substances (N-acetyl glucosamine and N-glycolymuramic acid in approximately equimolar amounts) as described in U.S. Patents 3,956,481 and 4,036,953. These compounds differ from the delipidated and deglycolipidated *M. vaccae* (DD-*M. vaccae*) of the present invention in the following aspects of their composition:

1. They are water-soluble agents, whereas DD-*M. vaccae* is insoluble in aqueous solutions.
2. They consist of a range of small oligomers of the mycobacterial cell wall unit, either extracted from bacteria by various solvents, or digested from the cell wall by an enzyme. In contrast, DD-*M. vaccae* contains highly polymerised cell wall.
3. All protein has been removed from their preparations by digestion with proteolytic enzymes. The only constituents of their preparations are the components of the cell wall peptidoglycan structure, namely alanine, glutamic acid, diaminopimelic acid, N-acetyl glucosamine, and N-glycolylmuramic acid. In contrast, DD-*M. vaccae* contains 50% w/w protein, comprising a number of distinct protein species.

The delivery of vaccines by nasal aerosols to reach lung tissue, or by oral delivery to the gastrointestinal tract has been generally limited to attenuated strains of virus. For example, vaccination against poliovirus has employed oral delivery of attenuated strains of this virus since the development of the Sabin vaccine. Aviron Incorporated and the National Institute of Allergy and Infectious Diseases in the United States have recently reported the

successful use of an influenza vaccine administered in a nasal spray. In this case, a live attenuated influenza strain provided 93% protection against influenza in young children. Vaccines consisting of killed viruses or bacteria, or of recombinant proteins have not been delivered by nasal aerosol or oral delivery. There are several reasons for this. There are few reports of successful immunisation resulting in T cell immunity or antibody synthesis employing these agents administered nasally. Further, oral delivery of proteins and killed organisms often results in the development of tolerance, which is exactly the reverse outcome sought in successful immunisation.

Sarcoidosis is a disease of unknown cause characterised by granulomatous inflammation affecting many organs of the body and especially the lungs, lymph nodes and liver. Sarcoid granulomata are composed of mononuclear phagocytes, with epithelioid and giant cells in their centre, and T lymphocytes. CD4 T lymphocytes are closely associated with the epithelioid cells while both CD4 and CD8 T lymphocytes accumulate at the periphery. The characteristic immunological abnormalities in sarcoidosis include peripheral blood and bronchoalveolar lavage hyper-globulinaemia and depression of 'delayed type' hypersensitivity reactions in the skin to tuberculin and other similar antigens, such as *Candida* and mumps. Peripheral blood lymphocyte numbers are reduced and CD4: CD8 ratios in peripheral blood are depressed to approximately 1-1.5:1. These are not manifestations of a generalised immune defect, but rather the consequence of heightened immunological activity which is 'compartmentalised' to sites of disease activity. In patients with pulmonary sarcoidosis, the total number of cells recovered by bronchoalveolar lavage is increased five- to ten-fold and the proportion of lymphocytes increased from the normal of less than 10-14% to between 15% and 50%. More than 90% of the lymphocytes recovered are T lymphocytes and the CD4:CD8 ratio has been reported to be increased from the value of 1.8:1 in normal controls to 10.5:1. The T lymphocytes are predominantly of the Th1 class, producing IFN- γ and IL-2 cytokines, rather than of the Th2 class. Following treatment, the increase in Th1 lymphocytes in sarcoid lungs is corrected.

Sarcoidosis involves the lungs in nearly all cases. Even when lesions are predominantly seen in other organs, subclinical lung involvement is usually present. While

some cases of sarcoidosis resolve spontaneously, approximately 50% of patients have at least a mild degree of permanent organ dysfunction. In severe cases, lung fibrosis develops and progresses to pulmonary failure requiring lung transplantation. The mainstay of treatment for sarcoidosis is corticosteroids. Patients initially responding to corticosteroids often relapse and require treatment with other immunosuppressive drugs such as methotrexate or cyclosporine.

Asthma is a common disease, with a high prevalence in the developed world. Asthma is characterised by increased responsiveness of the tracheobronchial tree to a variety of stimuli, the primary physiological disturbance being reversible airflow limitation, which may be spontaneous or drug-related, and the pathological hallmark being inflammation of the airways. Clinically, asthma can be subdivided into extrinsic and intrinsic variants.

Extrinsic asthma has an identifiable precipitant, and can be thought of as being atopic, occupational and drug-induced. Atopic asthma is associated with the enhancement of a Th2-type of immune response with the production of specific immunoglobulin E (IgE), positive skin tests to common aeroallergens and/or atopic symptoms. It can be divided further into seasonal and perennial forms according to the seasonal timing of symptoms. The airflow obstruction in extrinsic asthma is due to nonspecific bronchial hyperresponsiveness caused by inflammation of the airways. This inflammation is mediated by chemicals released by a variety of inflammatory cells including mast cells, eosinophils and lymphocytes. The actions of these mediators result in vascular permeability, mucus secretion and bronchial smooth muscle constriction. In atopic asthma, the immune response producing airway inflammation is brought about by the Th2 class of T cells which secrete IL-4, IL-5 and IL-10. It has been shown that lymphocytes from the lungs of atopic asthmatics produce IL-4 and IL-5 when activated. Both IL-4 and IL-5 are cytokines of the Th2 class and are required for the production of IgE and involvement of eosinophils in asthma. Occupational asthma may be related to the development of IgE to a protein hapten, such as acid anhydrides in plastic workers and plicatic acid in some western red cedar-induced asthma, or to non-IgE related mechanisms, such as that seen in toluene diisocyanate-induced asthma. Drug-induced asthma can be seen after the administration of aspirin or other non-steroidal anti-inflammatory drugs, most often in a certain subset of patients who may display other features such as nasal

polyposis and sinusitis. Intrinsic or cryptogenic asthma is reported to develop after upper respiratory tract infections, but can arise *de novo* in middle-aged or older people, in whom it is more difficult to treat than extrinsic asthma.

Asthma is ideally prevented by the avoidance of triggering allergens but this is not always possible nor are triggering allergens always easily identified. The medical therapy of asthma is based on the use of corticosteroids and bronchodilator drugs to reduce inflammation and reverse airway obstruction. In chronic asthma, treatment with corticosteroids leads to unacceptable adverse side effects.

Another disorder with a similar immune abnormality to asthma is allergic rhinitis. Allergic rhinitis is a common disorder and is estimated to affect at least 10% of the population. Allergic rhinitis may be seasonal (hay fever) caused by allergy to pollen. Non-seasonal or perennial rhinitis is caused by allergy to antigens such as those from house dust mite or animal dander.

The abnormal immune response in allergic rhinitis is characterised by the excess production of IgE antibodies specific against the allergen. The inflammatory response occurs in the nasal mucosa rather than further down the airways as in asthma. Like asthma, local eosinophilia in the affected tissues is a major feature of allergic rhinitis. As a result of this inflammation, patients develop sneezing, nasal discharge and congestion. In more severe cases, the inflammation extends to the eyes (conjunctivitis), palate and the external ear. While it is not life threatening, allergic rhinitis may be very disabling, prevent normal activities, and interfere with a person's ability to work. Current treatment involves the use of antihistamines, nasal decongestants and, as for asthma, sodium cromoglycate and corticosteroids.

Lung cancer is the leading cause of death from cancer. The incidence of lung cancer continues to rise and the World Health Organisation estimates that by 2000AD there will be 2 million new cases annually. Lung cancers may be broadly classified into two categories: small cell lung cancer (SCLC) which represents 20-25% of all lung cancers, and non-small cell lung cancer (NSCLC) which accounts for the remaining 75%. The majority of SCLC is caused by tobacco smoke. SCLC tends to spread early and 90% of patients present at diagnosis with involvement of the mediastinal lymph nodes in the chest. SCLC is treated by

chemotherapy, or a combination of chemotherapy and radiotherapy. Complete response rates vary from 10% to 50%. For the rare patient without lymph node involvement, surgery followed by chemotherapy may result in cure rates exceeding 60%. The prognosis for NSCLC is more dismal, as most patients have advanced disease by the time of diagnosis. Surgical removal of the tumor is possible in a very small number of patients and the five year survival rate for NSCLC is only 5-10%.

The factors leading to the development of lung cancer are complex and multiple. Environmental and genetic factors interact and cause sequential and incremental abnormalities which lead to uncontrolled cell proliferation, invasion of adjacent tissues and spread to distant sites.

Both cell-mediated and humoral immunity have been shown to be impaired in patients with lung cancer. Radiotherapy and chemotherapy further impair the immune function of patients. Attempts have been made to immunise patients with inactivated tumour cells or tumour antigens to enhance host anti-tumor response. Bacillus Calmette-Guerin (BCG) has been administered into the chest cavity following lung cancer surgery to augment non-specific immunity. Attempts have been made to enhance anti-tumor immunity by giving patients lymphocytes treated *ex vivo* with interleukin-2. These lymphokine-activated lymphocytes acquire the ability to kill tumor cells. The current immunotherapies for lung cancer are still at a developmental stage and their efficacies yet to be established for the standard management of lung cancer.

In one aspect, this invention deals with treatment of disorders of skin which appear to be associated with factors that influence the balance of thymus-derived (T) immune cells known as Th1 and Th2. These T cells are identified by their cytokine secretion phenotype. A common feature of treatment is the use of compounds prepared from *M. vaccae* which have immunomodulating properties that alter the balance of activities of these T cells as well as other immune cells.

Psoriasis is a common, chronic inflammatory skin disease which can be associated with various forms of arthritis in a minority of patients. The defect in psoriasis appears to be overly rapid growth of keratinocytes and shedding of scales from the skin surface. Drug

therapy is directed at slowing down this process. The disease may become manifest at any age. Spontaneous remission is relatively rare, and life-long treatment is usually necessary. Psoriasis produces chronic, scaling red patches on the skin surface. Psoriasis is a very visible disease, it frequently affects the face, scalp, trunk and limbs. The disease is emotionally and physically debilitating for the patient, detracting significantly from the quality of life. Between one and three million individuals in the United States have psoriasis with nearly a quarter million new cases occurring each year. Conservative estimates place the costs of psoriasis care in the United States currently at \$248 million a year.

There are two major hypotheses concerning the pathogenesis of psoriasis. The first is that genetic factors determine abnormal proliferation of epidermal keratinocytes. The cells no longer respond normally to external stimuli such as those involved in maintaining epidermal homeostasis. Abnormal expression of cell membrane cytokine receptors or abnormal transmembrane signal transduction might underlie cell hyperproliferation. Inflammation associated with psoriasis is secondary to the release of pro-inflammatory molecules from hyperproliferative keratinocytes.

A second hypothesis is that T cells interacting with antigen-presenting cells in skin release pro-inflammatory and keratinocyte-stimulating cytokines (Hancock, G.E. et al., *J. Exp. Med.* 168:1395-1402, 1988). Only T cells of genetically predetermined individuals possess the capacity to be activated under such circumstances. The keratinocytes themselves may be the antigen-presenting cell. The cellular infiltrate in psoriatic lesions show an influx of CD4+ T cells and, more prominently, CD8+ T cells (Bos, J.D. et al., *Arch. Dermatol. Res.* 281:23-3, 1989; Baker, B.S., *Br. J. Dermatol.* 110:555-564, 1984).

As the majority (90%) of psoriasis patients have limited forms of the disease, topical treatments which include dithranol, tar preparations, corticosteroids and the recently introduced vitamin D3 analogues (calcipotriol, calcitriol) can be used. A minority (10%) of psoriasis patients have a more serious condition, for which a number of systemic therapeutic modalities are available. Specific systemic therapies include UVB, PUVA, methotrexate, vitamin A derivatives (acitretin) and immuno-suppressants such as Cyclosporin A. The effectiveness of Cyclosporin and FK-506 for treating psoriasis provides support for the T cell

hypothesis as the prime cause of the disease (Bos, J.D. et al., *Lancet II*: 1500-1502, 1989; Ackerman, C. et al., *J. Invest. Dermatol.* 96:536 [abstract], 1991).

Atopic dermatitis is a chronic pruritic inflammatory skin disease which usually occurs in families with an hereditary predisposition for various allergic disorders such as allergic rhinitis and asthma. Atopic dermatitis occurs in approximately 10% of the general population. The main symptoms are dry skin, dermatitis (eczema) localised mainly in the face, neck and on the flexor sides and folds of the extremities accompanied by severe itching. It typically starts within the first two years of life. In about 90% of the patients this skin disease disappears during childhood but the symptoms can continue into adult life. It is one of the commonest forms of dermatitis world-wide. It is generally accepted that in atopy and in atopic dermatitis, a T cell abnormality is primary and that the dysfunction of T cells which normally regulate the production of IgE is responsible for the excessive production of this immunoglobulin.

Allergic contact dermatitis is a common non-infectious inflammatory disorder of the skin. In contact dermatitis, immunological reactions cannot develop until the body has become sensitised to a particular antigen. Subsequent exposure of the skin to the antigen and the recognition of these antigens by T cells result in the release of various cytokines, proliferation and recruitment of T cells, and finally in dermatitis (eczema).

Only a small proportion of the T cells in a lesion of allergic contact dermatitis are specific for the relevant antigen. Activated T cells probably migrate to the sites of inflammation regardless of antigen-specificity. Delayed-type hypersensitivity can only be transferred by T cells (CD4⁺ cells) sharing the MHC class II antigens. The 'response' to contact allergens can be transferred by T cells sharing either MHC class I (CD8⁺ cells) or class II (CD4⁺ cells) molecules (Sunday, M.E. et al., *J. Immunol.* 125:1601-1605, 1980). Keratinocytes can produce interleukin-1 which can facilitate the antigen presentation to T cells. The expression of the surface antigen intercellular adhesion molecule-1 (ICAM-1) is induced both on keratinocytes and endothelium by the cytokines tumor necrosis factor (TNF) and interferon-gamma (IFN- γ).

If the causes can be identified, removal alone will cure allergic contact dermatitis. During active inflammation, topical corticosteroids are useful. An inhibitory effect of cyclosporin has been observed in delayed-type hypersensitivity on the pro-inflammatory function(s) of primed T cells *in vitro* (Shidani, B. et al., *Eur. J. Immunol.* 14:314-318, 1984). The inhibitory effect of cyclosporin on the early phase of T cell activation in mice has also been reported (Milon, G. et al., *Ann. Immunol. (Inst. Pasteur)* 135d: 237-245, 1984).

Alopecia areata is a common hair disease, which accounts for about 2% of the consultations at dermatological outpatient clinics in the United States. The hallmark of this disease is the formation of well-circumscribed round or oval patches of non-scarring alopecia which may be located in any hairy area of the body. The disease may develop at any age. The onset is usually sudden and the clinical course is varied.

At present, it is not possible to attribute all or indeed any case of alopecia areata to a single cause (Rook, A. and Dawber, R, *Diseases of the Hair and Scalp*; Blackwell Scientific Publications 1982: 272-30). There are many factors that appear to be involved. These include genetic factors, atopy, association with disorders of supposed autoimmune etiology, Down's syndrome and emotional stress. The prevalence of atopy in patients with alopecia areata is increased. There is evidence that alopecia areata is an autoimmune disease. This evidence is based on consistent histopathological findings of a lymphocytic T cell infiltrate in and around the hair follicles with increased numbers of Langerhans cells, the observation that alopecia areata will respond to treatment with immunomodulating agents, and that there is a statistically significant association between alopecia areata and a wide variety of autoimmune diseases (Mitchell, A.J. et al., *J. Am. Acad. Dermatol.* 11:763-775, 1984).

Immunophenotyping studies on scalp biopsy specimens shows expression of HLA-DR on epithelial cells in the presumptive cortex and hair follicles of active lesions of alopecia areata, as well as a T cell infiltration with a high proportion of helper/inducer T cells in and around the hair follicles, increased numbers of Langerhans cells and the expression of ICAM-1 (Messenger, A.G. et al., *J. Invest. Dermatol.* 85:569-576, 1985; Gupta, A.K. et al., *J. Am. Acad. Dermatol.* 22:242-250, 1990).

The large variety of therapeutic modalities in alopecia areata can be divided into four categories: (i) non-specific topical irritants; (ii) 'immune modulators' such as systemic corticosteroids and PUVA; (iii) 'immune enhancers' such as contact dermatitis inducers, cyclosporin and inosiplex; and (iv) drugs of unknown action such as minoxidil (Dawber, R.P.R. et al., Textbook of Dermatology, Blackwell Scientific Publications, 5th Ed, 1982:2533-2638). Non-specific topical irritants such as dithranol may work through as yet unidentified mechanisms rather than local irritation in eliciting regrowth of hair. Topical corticosteroids may be effective but prolonged therapy is often necessary. Intralesional steroids have proved to be more effective but their use is limited to circumscribed patches of less active disease or to maintain regrowth of the eyebrows in alopecia totalis. Photochemotherapy has proved to be effective, possibly by changing functional subpopulations of T cells. Topical immunotherapy by means of induction and maintenance of allergic contact dermatitis on the scalp may result in hair regrowth in as many as 70% of the patients with alopecia areata. Diphenycprone is a potent sensitiser free from mutagenic activity. Oral cyclosporin can be effective in the short term (Gupta, A.K. et al., *J. Am. Acad. Dermatol.* 22:242-250, 1990). Inosiplex, an immunostimulant, has been used with apparent effectiveness in an open trial. Topical 5% minoxidil solution has been reported to be able to induce some hair growth in patients with alopecia areata. The mechanism of action is unclear.

Carcinomas of the skin are a major public health problem because of their frequency and the disability and disfigurement that they cause. Carcinoma of the skin is principally seen in individuals in their prime of life, especially in fair skinned individuals exposed to large amounts of sunlight. The annual cost of treatment and time loss from work exceeds \$250 million dollars a year in the United States alone. The three major types - basal cell cancer, squamous cell cancer, and melanoma - are clearly related to sunlight exposure.

Basal cell carcinomas are epithelial tumours of the skin. They appear predominantly on exposed areas of the skin. In a recent Australian study, the incidence of basal cell carcinomas was 652 new cases per year per 100,000 of the population. This compares with 160 cases of squamous cell carcinoma or 19 of malignant melanoma (Giles, G. et al., *Br. Med. J.* 296:13-17, 1988). Basal cell carcinomas are the most common of all cancers.

Lesions are usually surgically excised. Alternate treatments include retinoids, 5-fluorouracil, cryotherapy and radiotherapy. Alpha or gamma interferon have also been shown to be effective in the treatment of basal cell carcinomas, providing a valuable alternative to patients unsuitable for surgery or seeking to avoid surgical scars (Cornell et al., *J. Am. Acad. Dermatol.* 23:694-700, 1990; Edwards, L. et al., *J. Am. Acad. Dermatol.* 22:496-500, 1990).

Squamous cell carcinoma (SCC) is the second most common cutaneous malignancy, and its frequency is increasing. There are an increasing number of advanced and metastatic cases related to a number of underlying factors. Currently, metastatic SCC contributes to over 2000 deaths per year in the United States; the 5 year survival rate is 35%, with 90% of the metastases occurring by 3 years. Metastasis almost always occurs at the first lymphatic drainage station. The need for medical therapy for advanced cases is clear. A successful medical therapy for primary SCC of the skin would obviate the need for surgical excision with its potential for scarring and other side effects. This development may be especially desirable for facial lesions.

Because of their antiproliferative and immunomodulating effects *in vitro*, interferons (IFNs) have also been used in the treatment of melanoma (Kirkwood, J.M. et al., *J. Invest. Dermatol.* 95:180S-4S, 1990). Response rates achieved with systemic IFN- α , in either high or low dose, in metastatic melanoma were in the range 5-30%. Recently, encouraging results (30% response) were obtained with a combination of IFN- α and DTIC. Preliminary observations indicate a beneficial effect of IFN- α in an adjuvant setting in patients with high risk melanoma. Despite the low efficacy of IFN monotherapy in metastatic disease, several randomised prospective studies are now being performed with IFNs as an adjuvant or in combination with chemotherapy (McLeod, G.R. et al., *J. Invest. Dermatol.* 95:185S-7S, 1990; Ho, V.C. et al., *J. Invest. Dermatol.* 22:159-76, 1990).

Of all the available therapies for treating cutaneous viral lesions, only interferon possesses a specific antiviral mode of action, by reproducing the body's immune response to infection. Interferon treatment cannot eradicate the viruses however, although it may help with some manifestations of the infection. Interferon treatment is also associated with systemic adverse effects, requires multiple injections into each single wart and has a

significant economic cost (Kraus, S.J. et al., *Review of Infectious Diseases* 2(6):S620-S632, 1990; Frazer, I.H., *Current Opinion in Immunology* 8(4):484-491, 1996).

Summary of the Invention

Briefly stated, the present invention provides compositions present in or derived from *M. vaccae* and methods for their use in the prevention, treatment and diagnosis of diseases, including mycobacterial infection, immune disorders of the respiratory system, and skin disorders. The inventive methods comprise administering a composition having antigenic and/or adjuvant properties. Diseases of the respiratory system which may be treated using the inventive compositions include mycobacterial infections (such as infection with *M. tuberculosis* and/or *M. avium*), asthma, sarcoidosis and lung cancers. Disorders of the skin which may be treated using the inventive compositions include psoriasis, atopic dermatitis, allergic contact dermatitis, alopecia areata, and the skin cancers basal cell carcinoma, squamous cell carcinoma and melanoma. Adjuvants for use in vaccines or immunotherapy of infectious diseases and cancers are also provided.

In a first aspect, isolated polypeptides derived from *Mycobacterium vaccae* are provided comprising an immunogenic portion of an antigen, or a variant of such an antigen. In specific embodiments, the antigen includes an amino acid sequence selected from the group consisting of: (a) sequences recited in SEQ ID NO: 143, 145, 147, 152, 154 156, 158, 160, 162, 165, 166, 170, 172, 174, 177, 178, 181, 182, 184, 186, 187, 192, 194, 196, 197, 199, 201, 203, 205 and 207; (b) sequences having at least about 50% identical residues to a sequence recited in SEQ ID NO: 143, 145, 147, 152, 154 156, 158, 160, 162, 165, 166, 170, 172, 174, 177, 178, 181, 182, 184, 186, 187, 192, 194, 196, 197, 199, 201, 203, 205 and 207; (c) sequences having at least about 75% identical residues to a sequence recited in SEQ ID NO: 143, 145, 147, 152, 154 156, 158, 160, 162, 165, 166, 170, 172, 174, 177, 178, 181, 182, 184, 186, 187, 192, 194, 196, 197, 199, 201, 203, 205 and 207; and (d) sequences having at least about 95% identical residues to a sequence recited in SEQ ID NO: 143, 145, 147, 152, 154 156, 158, 160, 162, 165, 166, 170, 172, 174, 177, 178, 181, 182, 184, 186, 187, 192, 194, 196,

197, 199, 201, 203, 205 and 207, measured using alignments produced by the computer algorithm BLASTP, as described below.

DNA sequences encoding the inventive polypeptides, expression vectors comprising these DNA sequences, and host cells transformed or transfected with such expression vectors are also provided. In another aspect, the present invention provides fusion proteins comprising at least one polypeptide of the present invention.

Within other aspects, the present invention provides pharmaceutical compositions that comprise at least one of the inventive polypeptides, or a DNA molecule encoding such a polypeptide, and a physiologically acceptable carrier. The invention also provides vaccines comprising at least one of the above polypeptides, or at least one DNA sequence encoding such polypeptides, and a non-specific immune response amplifier. In certain embodiments, the non-specific immune response enhancer is selected from the group consisting of: delipidated and deglycolipidated *M. vaccae* cells; inactivated *M. vaccae* cells; delipidated and deglycolipidated *M. vaccae* cells depleted of mycolic acids; delipidated and deglycolipidated *M. vaccae* cells depleted of mycolic acids and arabinogalactan; and *M. vaccae* culture filtrate.

In yet another aspect, methods are provided for enhancing an immune response in a patient, comprising administering to a patient an effective amount of one or more of the above pharmaceutical compositions and/or vaccines. In one embodiment, the immune response is a Th1 response. In further aspects of this invention, methods are provided for the treatment of a disorder in a patient, comprising administering to the patient a pharmaceutical composition or vaccine of the present invention. In certain embodiments, the disorder is selected from the group consisting of immune disorders, infectious diseases, skin diseases and diseases of the respiratory system. Examples of such diseases include mycobacterial infections, asthma and psoriasis.

In other aspects, the invention provides methods for the treatment of immune disorders, infectious diseases, skin diseases or diseases of the respiratory system, comprising administering a composition comprising inactivated *M. vaccae* cells, delipidated and deglycolipidated *M. vaccae* cells or *M. vaccae* culture filtrate.

Methods for enhancing an immune response to an antigen are also provided. In one embodiment, such methods comprising administering a polypeptide that comprises an immunogenic portion of a *M. vaccae* antigen which includes a sequence of SEQ ID NO: 89 or 201, or a variant thereof. In a further embodiment, such methods comprise administering a composition comprising a component selected from the group consisting of: delipidated and deglycolipidated *M.vaccae* cells depleted of mycolic acids, and delipidated and deglycolipidated *M.vaccae* cells depleted of mycolic acids and arabinogalactan.

In further aspects of this invention, methods and diagnostic kits are provided for detecting mycobacterial infection in a patient. In a first embodiment, the method comprises contacting dermal cells of a patient with one or more of the above polypeptides and detecting an immune response on the patient's skin. In a second embodiment, the method comprises contacting a biological sample with at least one of the above polypeptides; and detecting in the sample the presence of antibodies that bind to the polypeptide or polypeptides, thereby detecting *M. tuberculosis* infection in the biological sample. Suitable biological samples include whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid and urine.

Diagnostic kits comprising one or more of the above polypeptides in combination with an apparatus sufficient to contact the polypeptide with the dermal cells of a patient are provided. The present invention also provides diagnostic kits comprising one or more of the inventive polypeptides in combination with a detection reagent.

In yet another aspect, the present invention provides antibodies, both polyclonal and monoclonal, that bind to the polypeptides described above, as well as methods for their use in the detection of *mycobacterial* infection.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

Brief Description of the Drawings

Figs. 1A and 1B illustrate the protective effects of immunizing mice with autoclaved *M. vaccae* or unfractionated *M. vaccae* culture filtrates, respectively, prior to infection with live *M. tuberculosis* H37Rv.

Figs. 2A and B show the percentage of eosinophils in mice immunized intranasally with either 10 or 1000 µg of heat-killed *M. vaccae* or 200-100 µg of DD-*M. vaccae*, respectively, 4 weeks prior to challenge with ovalbumin, as compared to control mice. Figs. 2C and D show the percentage of eosinophils in mice immunized intranasally with either 100 µg of heat-killed *M. vaccae* or 200 µg of DD-*M. vaccae*, respectively, as late as one week prior to challenge with ovalbumin. Fig. 2E shows the percentage of eosinophils in mice immunized either intranasally (i.n.) or subcutaneously (s.c.) with either BCG of the Pasteur strain (BCG-P), BCG of the Connought strain (BCG-C), 1 mg of heat-killed *M. vaccae*, or 200 µg of DD-*M. vaccae* prior to challenge with ovalbumin.

Fig. 3A illustrates the effect of immunizing mice with heat-killed *M. vaccae* or delipidated and deglycolipidated *M. vaccae* (DD-*M. vaccae*) prior to infection with tuberculosis. Fig. 3B illustrates the effect of immunizing mice with heat-killed *M. vaccae*, recombinant *M. vaccae* proteins, or a combination of heat-killed *M. vaccae* and *M. vaccae* recombinant proteins prior to infection with tuberculosis.

Fig. 4 illustrates the induction of IL-12 by autoclaved *M. vaccae*, lyophilized *M. vaccae*, delipidated and deglycolipidated *M. vaccae* and *M. vaccae* glycolipids.

Fig. 5 compares the *in vitro* stimulation of interferon-gamma production in spleen cells from Severe Combined ImmunoDeficient (SCID) mice by different concentrations of heat-killed (autoclaved) *M. vaccae*, delipidated and deglycolipidated *M. vaccae*, and *M. vaccae* glycolipids.

Figs. 6A, B and C illustrate the stimulation of interferon-gamma production by different concentrations of *M. vaccae* recombinant proteins, heat-killed *M. vaccae*, delipidated and deglycolipidated *M. vaccae* (referred to in the figure as "delipidated *M. vaccae*"), *M. vaccae* glycolipids and lipopolysaccharide, in peritoneal macrophages from C57BL/6 mice (Fig. 6A), BALB/C mice (Fig. 6B) or C3H/HeJ mice (Fig. 6C).

Fig. 7A(i) - (iv) illustrate the non-specific immune amplifying effects of 10 μ g, 100 μ g and 1mg autoclaved *M. vaccae* and 75 μ g unfractionated culture filtrates of *M. vaccae*, respectively. Fig. 7B(i) and (ii) illustrate the non-specific immune amplifying effects of autoclaved *M. vaccae*, and delipidated and deglycolipidated *M. vaccae*, respectively. Fig. 7C(i) illustrates the non-specific immune amplifying effects of whole autoclaved *M. vaccae*. Fig. 7C(ii) illustrates the non-specific immune amplifying effects of soluble *M. vaccae* proteins extracted with SDS from delipidated and deglycolipidated *M. vaccae*. Fig. 7C(iii) illustrates that the non-specific amplifying effects of the preparation of Fig. 7C(ii) are destroyed by treatment with the proteolytic enzyme Pronase. Fig. 7D illustrates the non-specific immune amplifying effects of heat-killed *M. vaccae* (Fig. 7D(i)), whereas a non-specific immune amplifying effect was not seen with heat-killed preparations of *M. tuberculosis* (Fig. 7D(ii)), *M. bovis* BCG (Fig. 7D(iii)), *M. phlei* (Fig. 7D(iv)) and *M. smegmatis* (Fig. 7D(v)).

Figs. 8A and B illustrate the stimulation of CD69 expression on $\alpha\beta$ T cells, $\gamma\delta$ T cells and NK cells, respectively, by the *M. vaccae* protein GV23, the Th1-inducing adjuvants MPL/TDM/CWS and CpG ODN, and the Th2-inducing adjuvants aluminium hydroxide and cholera toxin.

Figs. 9A-D illustrate the effect of heat-killed *M. vaccae*, DD-*M. vaccae* and *M. vaccae* recombinant proteins on the production of IL-1 β , TNF- α , IL-12 and IFN- γ , respectively, by human PBMC.

Figs. 10A-C illustrate the effects of varying concentrations of the recombinant *M. vaccae* proteins GV-23 and GV-45 on the production of IL-1 β , TNF- α and IL-12, respectively, by human PBMC.

Figs. 11A-D illustrate the stimulation of IL-1 β , TNF- α , IL-12 and IFN- γ production, respectively, in human PBMC by the *M. vaccae* protein GV23, the Th1-inducing adjuvants MPL/TDM/CWS and CpG ODN, and the Th2-inducing adjuvants aluminium hydroxide and cholera toxin.

Figs. 12A-C illustrate the effects of varying concentrations of the recombinant *M. vaccae* proteins GV-23 and GV-45 on the expression of CD40, CD80 and CD86, respectively, by dendritic cells.

Fig. 13 illustrates the enhancement of dendritic cell mixed leukocyte reaction by the recombinant *M. vaccae* protein GV-23.

Detailed Description of the Invention

As noted above, the present invention is generally directed to compositions and methods for preventing, treating and diagnosing infectious diseases and immune disorders. Disorders which may be effectively treated using the inventive compositions include diseases of the respiratory system, such as mycobacterial infections, asthma, sarcoidosis and lung cancers, and disorders of the skin, such as psoriasis, atopic dermatitis, allergic contact dermatitis, alopecia areata, and the skin cancers basal cell carcinoma, squamous cell carcinoma and melanoma.

Effective vaccines that provide protection against infectious microorganisms contain at least two functionally different components. The first is an antigen, which may be polypeptide or carbohydrate in nature, and which is processed by macrophages and other antigen-presenting cells and displayed for CD4⁺ T cells or for CD8⁺ T cells. This antigen forms the "specific" target of an immune response. The second component of a vaccine is a non-specific immune response amplifier, termed an adjuvant, with which the antigen is mixed or is incorporated into. An adjuvant amplifies either cell-mediated or antibody immune responses to a structurally unrelated compound or polypeptide. Several known adjuvants are prepared from microbes such as *Bordetella pertussis*, *M. tuberculosis* and *M. bovis* BCG. Adjuvants may also contain components designed to protect polypeptide antigens from degradation, such as aluminum hydroxide or mineral oil. While the antigenic component of a vaccine contains polypeptides that direct the immune attack against a specific pathogen, such as *M. tuberculosis*, the adjuvant is often capable of broad use in many different vaccine formulations. Certain known proteins, such as bacterial enterotoxins, can function both as an

antigen to elicit a specific immune response and as an adjuvant to enhance immune responses to unrelated proteins.

Certain pathogens, such as *M. tuberculosis*, as well as certain cancers, are effectively contained by an immune attack directed by CD4⁺ and CD8⁺ T cells, known as cell-mediated immunity. Other pathogens, such as poliovirus, also require antibodies, produced by B cells, for containment. These different classes of immune attack (T cell or B cell) are controlled by different subpopulations of CD4⁺ T cells, commonly referred to as Th1 and Th2 cells. A desirable property of an adjuvant is the ability to selectively amplify the function of either Th1 or Th2 populations of CD4⁺ T cells. Many skin disorders, including psoriasis, atopic dermatitis, alopecia, and skin cancers appear to be influenced by differences in the activity of these Th cell subsets.

The two types of Th cell subsets have been well characterized in a murine model and are defined by the cytokines they release upon activation. The Th1 subset secretes IL-2, IFN- γ and tumor necrosis factor, and mediates macrophage activation and delayed-type hypersensitivity response. The Th2 subset releases IL-4, IL-5, IL-6 and IL-10, which stimulate B cell activation. The Th1 and Th2 subsets are mutually inhibiting, so that IL-4 inhibits Th1-type responses, and IFN- γ inhibits Th2-type responses. Similar Th1 and Th2 subsets have been found in humans, with release of the identical cytokines observed in the murine model. In particular, the majority of T-cell clones from atopic human lymphocytes resemble the murine Th2 cell that produces IL-4, whereas very few clones produce IFN- γ . Therefore, the selective expression of the Th2 subset with subsequent production of IL-4 and decreased levels of IFN- γ -producing cells could lead to preferential enhancement of IgE production. Amplification of Th1-type immune responses is central to a reversal of disease state in many disorders, including disorders of the respiratory system such as tuberculosis, sarcoidosis, asthma, allergic rhinitis and lung cancers.

Inactivated *M. vaccae* and many compounds derived from *M. vaccae* have both antigen and adjuvant properties which function to enhance Th1-type immune responses. The methods of the present invention employ one or more of these antigen and adjuvant compounds from *M. vaccae* and/or its culture filtrates to redirect immune activities of T cells

in patients. Mixtures of such compounds are particularly effective in the methods disclosed herein. While it is well known that all mycobacteria contain many cross-reacting antigens, it is not known whether they contain adjuvant compounds in common. As shown below, inactivated *M. vaccae* and a modified (delipidated and deglycolipidated) form of inactivated *M. vaccae* have been found to have adjuvant properties of the Th1-type which are not shared by a number of other mycobacterial species. Furthermore, it has been found that *M. vaccae* produces compounds in its own culture filtrate which amplify the immune response to *M. vaccae* antigens also found in culture filtrate, as well as to antigens from other sources.

In one aspect, the present invention provides methods for the immunotherapy of respiratory and/or lung disorders, including tuberculosis, sarcoidosis, asthma, allergic rhinitis and lung cancers, in a patient to enhance Th1-type immune responses. In one embodiment, the compositions are delivered directly to the mucosal surfaces of airways leading to and/or within the lungs. However, the compositions may also be administered via intradermal or subcutaneous routes. Compositions which may be usefully employed in such methods comprise at least one of the following components: inactivated *M. vaccae* cells; *M. vaccae* culture filtrate; delipidated and deglycolipidated *M. vaccae* cells (DD-*M. vaccae*); and compounds present in or derived from *M. vaccae* and/or its culture filtrate. As illustrated below, administration of such compositions, results in specific T cell immune responses and enhanced protection against *M. tuberculosis* infection, and is also effective in the treatment of asthma. While the precise mode of action of these compositions in the treatment of diseases such as asthma is unknown, they are believed to suppress an asthma-inducing Th2 immune response.

As used herein the term "respiratory system" refers to the lungs, nasal passageways, trachea and bronchial passageways.

As used herein the term "airways leading to or located in the lung" includes the nasal passageways, mouth, tonsil tissue, trachea and bronchial passageways.

As used herein, a "patient" refers to any warm-blooded animal, preferably a human. Such a patient may be afflicted with disease or may be free of detectable disease. In other

words, the inventive methods may be employed to induce protective immunity for the prevention or treatment of disease.

In another aspect, the present invention provides methods for the immunotherapy of skin disorders, including psoriasis, atopic dermatitis, alopecia, and skin cancers in patients, in which immunotherapeutic agents are employed to alter or redirect an existing state of immune activity by altering the function of T cells to a Th1-type of immune response. Compositions which may be usefully employed in the inventive methods comprise at least one of the following components: inactivated *M. vaccae* cells; *M. vaccae* culture filtrate; modified *M. vaccae* cells; and constituents and compounds present in or derived from *M. vaccae* and/or its culture filtrate. As detailed below, multiple administrations of such compositions, preferably by intradermal injection, have been shown to be highly effective in the treatment of psoriasis.

As used herein the term "inactivated *M. vaccae*" refers to *M. vaccae* that have either been killed by means of heat, as detailed below in Example 7, or subjected to radiation, such as ⁶⁰Cobalt at a dose of 2.5 megarads. As used herein, the term "modified *M. vaccae*" includes delipidated *M. vaccae* cells, deglycolipidated *M. vaccae* cells and *M. vaccae* cells that have been both delipidated and deglycolipidated (DD-*M. vaccae*).

The preparation of DD-*M. vaccae* and its chemical composition are described below in Example 7. As detailed below, the inventors have shown that removal of the glycolipid constituents from *M. vaccae* results in the removal of molecular components that stimulate interferon-gamma production in natural killer (NK) cells, thereby significantly reducing the non-specific production of a cytokine that has numerous harmful side-effects.

In yet a further aspect, the present invention provides isolated polypeptides that comprise at least one immunogenic portion of a *M. vaccae* antigen, or a variant thereof, or at least one adjuvant portion of an *M. vaccae* protein. In specific embodiments, such polypeptides comprise an immunogenic portion of an antigen, or a variant thereof, wherein the antigen includes a sequence selected from the group consisting of SEQ ID NO: 1-4, 9-16, 18-21, 23, 25, 26, 28, 29, 44, 45, 47, 52-55, 63, 64, 70, 75, 89, 94, 98, 100-105, 109, 110, 112, 121, 124, 125, 134, 135, 140, 141, 143, 145, 147, 152, 154, 156, 158, 160, 165, 166, 170, 172, 174, 177, 178, 181, 182, 184, 186, 187, 192, 194, 201, 203, 205 and 207.

As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins (i.e., antigens), wherein the amino acid residues are linked by covalent peptide bonds. Thus, a polypeptide comprising an immunogenic portion of one of the above antigens may consist entirely of the immunogenic portion, or may contain additional sequences. The additional sequences may be derived from the native *M. vaccae* antigen or may be heterologous, and such sequences may (but need not) be immunogenic. As detailed below, polypeptides of the present invention may be isolated from *M. vaccae* cells or culture filtrate, or may be prepared by synthetic or recombinant means.

"Immunogenic," as used herein, refers to the ability to elicit an immune response in a patient, such as a human, or in a biological sample. In particular, immunogenic antigens are capable of stimulating cell proliferation, interleukin-12 production or interferon- γ production in biological samples comprising one or more cells selected from the group of T cells, NK cells, B cells and macrophages, where the cells are derived from an *M. tuberculosis*-immune individual. Exposure to an immunogenic antigen generally results in the generation of immune memory such that upon re-exposure to that antigen, an enhanced and more rapid response occurs.

Immunogenic portions of the antigens described herein may be prepared and identified using well known techniques, such as those summarised in Paul, *Fundamental Immunology*, 3d ed., Raven Press, 1993, pp. 243-247. Such techniques include screening polypeptide portions of the native antigen or protein for immunogenic properties. The representative proliferation and cytokine production assays described herein may be employed in these screens. An immunogenic portion of an antigen is a portion that, within such representative assays, generates an immune response (e.g., cell proliferation, interferon- γ production or interleukin-12 production) that is substantially similar to that generated by the full-length antigen. In other words, an immunogenic portion of an antigen may generate at least about 20%, preferably about 65%, and most preferably about 100% of the proliferation induced by the full-length antigen in the model proliferation assay described herein. An immunogenic portion may also, or alternatively, stimulate the production of at least about 20%, preferably

about 65% and most preferably about 100%, of the interferon- γ and/or interleukin-12 induced by the full length antigen in the model assay described herein.

A *M. vaccae* adjuvant is a compound found in *M. vaccae* cells or *M. vaccae* culture filtrates which non-specifically stimulates immune responses. Adjuvants enhance the immune response to immunogenic antigens and the process of memory formation. In the case of *M. vaccae* proteins, these memory responses favour Th1-type immunity. Adjuvants are also capable of stimulating interleukin-12 production or interferon- γ production in biological samples comprising one or more cells selected from the group of T cells, NK cells, B cells and macrophages, where the cells are derived from healthy individuals. Adjuvants may or may not stimulate cell proliferation. Such *M. vaccae* adjuvants include, for example, polypeptides comprising a sequence recited in SEQ ID NO: 89, 117, 160, 162 or 201.

The term "polynucleotide(s)," as used herein, means a single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases and includes DNA and corresponding RNA molecules, including HnRNA and mRNA molecules, both sense and anti-sense strands, and comprehends cDNA, genomic DNA and recombinant DNA, as well as wholly or partially synthesized polynucleotides. An HnRNA molecule contains introns and corresponds to a DNA molecule in a generally one-to-one manner. An mRNA molecule corresponds to an HnRNA and DNA molecule from which the introns have been excised. A polynucleotide may consist of an entire gene, or any portion thereof. Operable anti-sense polynucleotides may comprise a fragment of the corresponding polynucleotide, and the definition of "polynucleotide" therefore includes all such operable anti-sense fragments.

The compositions and methods of this invention also encompass variants of the above polypeptides and polynucleotides. As used herein, the term "variant" covers any sequence which has at least about 40%, more preferably at least about 60%, more preferably yet at least about 75% and most preferably at least about 90% identical residues (either nucleotides or amino acids) to a sequence of the present invention. The percentage of identical residues is determined by aligning the two sequences to be compared, determining the number of identical residues in the aligned portion, dividing that number by the total length of the inventive, or queried, sequence and multiplying the result by 100.

Polynucleotide or polypeptide sequences may be aligned, and percentage of identical nucleotides in a specified region may be determined against another polynucleotide, using computer algorithms that are publicly available. Two exemplary algorithms for aligning and identifying the similarity of polynucleotide sequences are the BLASTN and FASTA algorithms. The similarity of polypeptide sequences may be examined using the BLASTP algorithm. Both the BLASTN and BLASTP software are available on the NCBI anonymous FTP server (<ftp://ncbi.nlm.nih.gov>) under `/blast/executables/`. The BLASTN algorithm version 2.0.4 [Feb-24-1998], set to the default parameters described in the documentation and distributed with the algorithm, is preferred for use in the determination of variants according to the present invention. The use of the BLAST family of algorithms, including BLASTN and BLASTP, is described at NCBI's website at URL <http://www.ncbi.nlm.nih.gov/BLAST/newblast.html> and in the publication of Altschul, Stephen F., et al. (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", *Nucleic Acids Res.* 25:3389-3402. The computer algorithm FASTA is available on the Internet at the ftp site <ftp://ftp.virginia.edu/pub/fasta/>. Version 2.0u4, February 1996, set to the default parameters described in the documentation and distributed with the algorithm, is preferred for use in the determination of variants according to the present invention. The use of the FASTA algorithm is described in W.R. Pearson and D.J. Lipman, "Improved Tools for Biological Sequence Analysis," *Proc. Natl. Acad. Sci. USA* 85:2444-2448 (1988) and W.R. Pearson, "Rapid and Sensitive Sequence Comparison with FASTP and FASTA," *Methods in Enzymology* 183:63-98 (1990).

The following running parameters are preferred for determination of alignments and similarities using BLASTN that contribute to the E values and percentage identity: Unix running command: `blastall -p blastn -d embldb -e 10 -G 1 -E 1 -r 2 -v 50 -b 50 -i queryseq -o results`; and parameter default values:

- p Program Name [String]
- d Database [String]
- e Expectation value (E) [Real]
- G Cost to open a gap (zero invokes default behavior) [Integer]

- E Cost to extend a gap (zero invokes default behavior) [Integer]
- r Reward for a nucleotide match (blastn only) [Integer]
- v Number of one-line descriptions (V) [Integer]
- b Number of alignments to show (B) [Integer]
- i Query File [File In]
- o BLAST report Output File [File Out] Optional

For BLASTP the following running parameters are preferred: `blastall -p blastp -d swissprot -e 10 -G 1 -E 1 -v 50 -b 50 -i queryseq -o results`

- p Program Name [String]
- d Database [String]
- e Expectation value (E) [Real]
- G Cost to open a gap (zero invokes default behavior) [Integer]
- E Cost to extend a gap (zero invokes default behavior) [Integer]
- v Number of one-line descriptions (v) [Integer]
- b Number of alignments to show (b) [Integer]
- I Query File [File In]
- o BLAST report Output File [File Out] Optional

The "hits" to one or more database sequences by a queried sequence produced by BLASTN, BLASTP, FASTA, or a similar algorithm, align and identify similar portions of sequences. The hits are arranged in order of the degree of similarity and the length of sequence overlap. Hits to a database sequence generally represent an overlap over only a fraction of the sequence length of the queried sequence.

The BLASTN and FASTA algorithms also produce "Expect" values for alignments. The Expect value (E) indicates the number of hits one can "expect" to see over a certain number of contiguous sequences by chance when searching a database of a certain size. The Expect value is used as a significance threshold for determining whether the hit to a database, such as the preferred EMBL database, indicates true similarity. For example, an E value of 0.1 assigned to a hit is interpreted as meaning that in a database of the size of the EMBL database, one might expect to see 0.1 matches over the aligned portion of the sequence with a

similar score simply by chance. By this criterion, the aligned and matched portions of the sequences then have a probability of 90% of being the same. For sequences having an E value of 0.01 or less over aligned and matched portions, the probability of finding a match by chance in the EMBL database is 1% or less using the BLASTN or FASTA algorithm.

According to one embodiment, "variant" polynucleotides, with reference to each of the polynucleotides of the present invention, preferably comprise sequences having the same number or fewer nucleic acids than each of the polynucleotides of the present invention and producing an E value of 0.01 or less when compared to the polynucleotide of the present invention. That is, a variant polynucleotide is any sequence that has at least a 99% probability of being the same as the polynucleotide of the present invention, measured as having an E value of 0.01 or less using the BLASTN or FASTA algorithms set at the default parameters. According to a preferred embodiment, a variant polynucleotide is a sequence having the same number or fewer nucleic acids than a polynucleotide of the present invention that has at least a 99% probability of being the same as the polynucleotide of the present invention, measured as having an E value of 0.01 or less using the BLASTN or FASTA algorithms set at the default parameters.

Variant polynucleotide sequences will generally hybridize to the recited polynucleotide sequence under stringent conditions. As used herein, "stringent conditions" refers to prewashing in a solution of 6X SSC, 0.2% SDS; hybridizing at 65 °C, 6X SSC, 0.2% SDS overnight; followed by two washes of 30 minutes each in 1X SSC, 0.1% SDS at 65 °C and two washes of 30 minutes each in 0.2X SSC, 0.1% SDS at 65 °C.

Portions and other variants of *M. vaccae* polypeptides may be generated by synthetic or recombinant means. Synthetic polypeptides having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may be generated using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. See Merrifield, *J. Am. Chem. Soc.* 85:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied

BioSystems, Inc. (Foster City, CA), and may be operated according to the manufacturer's instructions. Variants of a native antigen or adjuvant may be prepared using standard mutagenesis techniques, such as oligonucleotide-directed site specific mutagenesis. Sections of the DNA sequence may also be removed using standard techniques to permit preparation of truncated polypeptides.

A polypeptide of the present invention may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (e.g., poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

In general, *M. vaccae* antigens, and DNA sequences encoding such antigens, may be prepared using any of a variety of procedures. For example, soluble antigens may be isolated from *M. vaccae* culture filtrate as described below. Antigens may also be produced recombinantly by inserting a DNA sequence that encodes the antigen into an expression vector and expressing the antigen in an appropriate host. Any of a variety of expression vectors known to those of ordinary skill in the art may be employed. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast and higher eukaryotic cells. Preferably, the host cells employed are *E. coli*, mycobacteria, insect, yeast or a mammalian cell line such as COS or CHO. The DNA sequences expressed in this manner may encode naturally occurring antigens, portions of naturally occurring antigens, or other variants thereof.

DNA sequences encoding *M. vaccae* antigens may be obtained by screening an appropriate *M. vaccae* cDNA or genomic DNA library for DNA sequences that hybridize to degenerate oligonucleotides derived from partial amino acid sequences of isolated soluble antigens. Suitable degenerate oligonucleotides may be designed and synthesized, and the screen may be performed as described, for example in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989. As

described below, polymerase chain reaction (PCR) may be employed to isolate a nucleic acid probe from genomic DNA, or a cDNA or genomic DNA library. The library screen may then be performed using the isolated probe. DNA molecules encoding *M. vaccae* antigens may also be isolated by screening an appropriate *M. vaccae* expression library with anti-sera (*e.g.*, rabbit or monkey) raised specifically against *M. vaccae* antigens.

Regardless of the method of preparation, the antigens described herein have the ability to induce an immunogenic response. More specifically, the antigens have the ability to induce cell proliferation and/or cytokine production (for example, interferon- γ and/or interleukin-12 production) in T cells, NK cells, B cells or macrophages derived from an *M. tuberculosis*-immune individual. An *M. tuberculosis*-immune individual is one who is considered to be resistant to the development of tuberculosis by virtue of having mounted an effective T cell response to *M. tuberculosis*. Such individuals may be identified based on a strongly positive (*i.e.*, greater than about 10 mm diameter induration) intradermal skin test response to tuberculosis proteins (PPD), and an absence of any symptoms of tuberculosis infection.

Assays for cell proliferation or cytokine production in T cells, NK cells, B cells or macrophages may be performed, for example, using the procedures described below. The selection of cell type for use in evaluating an immunogenic response to an antigen will depend on the desired response. For example, interleukin-12 production is most readily evaluated using preparations containing T cells, NK cells, B cells and macrophages derived from *M. tuberculosis*-immune individuals may be prepared using methods well known in the art. For example, a preparation of peripheral blood mononuclear cells (PBMCs) may be employed without further separation of component cells. PBMCs may be prepared, for example, using density centrifugation through Ficoll™ (Winthrop Laboratories, NY). T cells for use in the assays described herein may be purified directly from PBMCs. Alternatively, an enriched T cell line reactive against mycobacterial proteins, or T cell clones reactive to individual mycobacterial proteins, may be employed. Such T cell clones may be generated by, for example, culturing PBMCs from *M. tuberculosis*-immune individuals with mycobacterial proteins for a period of 2-4 weeks. This allows expansion of only the mycobacterial protein-specific T cells, resulting in a line composed solely of such cells. These cells may then be

cloned and tested with individual proteins, using methods well known in the art, to more accurately define individual T cell specificity.

In general, regardless of the method of preparation, the polypeptides disclosed herein are prepared in an isolated, substantially pure, form. Preferably, the polypeptides are at least about 80% pure, more preferably at least about 90% pure and most preferably at least about 99% pure. In certain preferred embodiments, described in detail below, the substantially pure polypeptides are incorporated into pharmaceutical compositions or vaccines for use in one or more of the methods disclosed herein.

The present invention also provides fusion proteins comprising a first and a second inventive polypeptide or, alternatively, a polypeptide of the present invention and a known *M tuberculosis* antigen, such as the 38 kDa antigen described in Andersen and Hansen, *Infect. Immun.* 57:2481-2488, 1989, together with variants of such fusion proteins. The fusion proteins of the present invention may also include a linker peptide between the first and second polypeptides.

A DNA sequence encoding a fusion protein of the present invention is constructed using known recombinant DNA techniques to assemble separate DNA sequences encoding the first and second polypeptides into an appropriate expression vector. The 3' end of a DNA sequence encoding the first polypeptide is ligated, with or without a peptide linker, to the 5' end of a DNA sequence encoding the second polypeptide so that the reading frames of the sequences are in phase to permit mRNA translation of the two DNA sequences into a single fusion protein that retains the biological activity of both the first and the second polypeptides.

A peptide linker sequence may be employed to separate the first and the second polypeptides by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the fusion protein using standard techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide

linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea et al., *Gene* 40:39-46, 1985; Murphy et al., *Proc. Natl. Acad. Sci. USA* 83:8258-8262, 1986; U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180. The linker sequence may be from 1 to about 50 amino acids in length. Peptide linker sequences are not required when the first and second polypeptides have non-essential N-terminal amino acid regions that can be used to separate the functional domains and prevent steric interference. The ligated DNA sequences encoding the fusion proteins are cloned into suitable expression systems using techniques known to those of ordinary skill in the art.

As detailed below, the inventors have demonstrated that heat-killed *M. vaccae*, DD-*M. vaccae* and recombinant *M. vaccae* proteins of the present invention may be employed to activate T cells and NK cells; to stimulate the production of cytokines (in particular Th1 class of cytokines) in human PBMC; to enhance the expression of co-stimulatory molecules on dendritic cells and monocytes (thereby enhancing activation); and to enhance dendritic cell maturation and function. Furthermore, the inventors have demonstrated similarities between the immunological properties of the inventive *M. vaccae* protein GV-23 and those of two known Th1-inducing adjuvants. GV-23 may thus be employed in the treatment of diseases that involve enhancing a Th1 immune response. Examples of such diseases include allergic diseases (for example, asthma and eczema) autoimmune diseases (for example, systemic lupus erythematosus) and infectious diseases (for example, tuberculosis and leprosy). In addition, GV-23 may be employed as a dendritic cell or NK cell enhancer in the treatment of immune deficiency disorders, such as HIV, and to enhance immune responses and cytotoxic responses to, for example, malignant cells in cancer and following immunosuppressive anti-cancer therapies, such as chemotherapy.

For use in the inventive therapeutic methods, the inactivated *M. vaccae*, *M. vaccae* culture filtrate, modified *M. vaccae* cells, *M. vaccae* polypeptide, fusion protein (or polynucleotides encoding such polypeptides or fusion proteins) is generally present within a pharmaceutical composition or a vaccine. Pharmaceutical compositions may comprise one or

more components selected from the group consisting of inactivated *M. vaccae* cells, *M. vaccae* culture filtrate, modified *M. vaccae* cells, and compounds present in or derived from *M. vaccae* and/or its culture filtrate, together with a physiologically acceptable carrier. Vaccines may comprise one or more components selected from the group consisting of inactivated *M. vaccae* cells, *M. vaccae* culture filtrate, modified *M. vaccae* cells, and compounds present in or derived from *M. vaccae* and/or its culture filtrate, together with a non-specific immune response amplifier. Such pharmaceutical compositions and vaccines may also contain other mycobacterial antigens, either, as discussed above, incorporated into a fusion protein or present within a separate polypeptide.

Alternatively, a vaccine of the present invention may contain DNA encoding one or more polypeptides as described above, such that the polypeptide is generated *in situ*. In such vaccines, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacterial and viral expression systems. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminator signal). Bacterial delivery systems involve the administration of a bacterium (such as *Bacillus Calmette-Guerin*) that expresses an immunogenic portion of the polypeptide on its cell surface. In a preferred embodiment, the DNA may be introduced using a viral expression system (e.g., vaccinia or other poxvirus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic, or defective, replication competent virus. Techniques for incorporating DNA into such expression systems are well known in the art. The DNA may also be "naked," as described, for example, in Ulmer et al., *Science* 259:1745-1749, 1993 and reviewed by Cohen, *Science* 259:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

A DNA vaccine as described above may be administered simultaneously with or sequentially to either a polypeptide of the present invention or a known mycobacterial antigen, such as the 38 kDa antigen described above. For example, administration of DNA encoding a polypeptide of the present invention, may be followed by administration of an antigen in order to enhance the protective immune effect of the vaccine.

Routes and frequency of administration, as well as dosage, will vary from individual to individual and may parallel those currently being used in immunization using BCG. In general, the pharmaceutical compositions and vaccines may be administered by injection (*e.g.*, intradermal, intramuscular, intravenous or subcutaneous), intranasally (*e.g.*, by aspiration) or orally. Between 1 and 3 doses may be administered for a 1-36 week period. Preferably, 3 doses are administered, at intervals of 3-4 months, and booster vaccinations may be given periodically thereafter. Alternate protocols may be appropriate for individual patients. A suitable dose is an amount of polypeptide or DNA that, when administered as described above, is capable of raising an immune response in a patient sufficient to protect the patient from mycobacterial infection for at least 1-2 years. In general, the amount of polypeptide present in a dose (or produced *in situ* by the DNA in a dose) ranges from about 1 pg to about 100 mg per kg of host, typically from about 10 pg to about 1 mg, and preferably from about 100 pg to about 1 μ g. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 ml to about 5 ml.

In one embodiment, the pharmaceutical composition or vaccine is in a form suitable for delivery to the mucosal surfaces of the airways leading to or within the lungs. For example, the pharmaceutical composition or vaccine may be suspended in a liquid formulation for delivery to a patient in an aerosol form or by means of a nebulizer device similar to those currently employed in the treatment of asthma. In other embodiments, the pharmaceutical composition or vaccine is in a form suitable for administration by injection (intracutaneous, intramuscular, intravenous or subcutaneous) or orally. While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will depend on the suitability for the chosen route of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a lipid, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (*e.g.*, polylactic galactide) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable

biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

Any of a variety of adjuvants may be employed in the vaccines of this invention to non-specifically enhance the immune response. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a non-specific stimulator of immune responses, such as lipid A, *Bordetella pertussis*, *M. tuberculosis*, or, as discussed below, *M. vaccae*. Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Freund's Complete Adjuvant (Difco Laboratories, Detroit, MI), and Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ). Other suitable adjuvants include alum, biodegradable microspheres, monophosphoryl lipid A and Quil A.

In another aspect, this invention provides methods for using one or more of the inventive polypeptides to diagnose tuberculosis using a skin test. As used herein, a "skin test" is any assay performed directly on a patient in which a delayed-type hypersensitivity (DTH) reaction (such as swelling, reddening or dermatitis) is measured following intradermal injection of one or more polypeptides as described above. Preferably, the reaction is measured at least 48 hours after injection, more preferably 48-72 hours.

The DTH reaction is a cell-mediated immune response, which is greater in patients that have been exposed previously to the test antigen (*i.e.*, the immunogenic portion of the polypeptide employed, or a variant thereof). The response may be measured visually, using a ruler. In general, a response that is greater than about 0.5 cm in diameter, preferably greater than about 1.0 cm in diameter, is a positive response, indicative of tuberculosis infection.

For use in a skin test, the polypeptides of the present invention are preferably formulated, as pharmaceutical compositions containing a polypeptide and a physiologically acceptable carrier, as described above. Such compositions typically contain one or more of the above polypeptides in an amount ranging from about 1 μ g to about 100 μ g, preferably from about 10 μ g to about 50 μ g in a volume of 0.1 ml. Preferably, the carrier employed in such pharmaceutical compositions is a saline solution with appropriate preservatives, such as phenol and/or Tween 80™.

In a preferred embodiment, a polypeptide employed in a skin test is of sufficient size such that it remains at the site of injection for the duration of the reaction period. In general, a polypeptide that is at least 9 amino acids in length is sufficient. The polypeptide is also preferably broken down by macrophages or dendritic cells within hours of injection to allow presentation to T-cells. Such polypeptides may contain repeats of one or more of the above sequences or other immunogenic or nonimmunogenic sequences.

In another aspect, methods are provided for detecting mycobacterial infection in a biological sample, using one or more of the inventive polypeptides, either alone or in combination. In embodiments in which multiple polypeptides are employed, polypeptides other than those specifically described herein, such as the 38 kDa antigen described above, may be included. As used herein, a "biological sample" is any antibody-containing sample obtained from a patient. Preferably, the sample is whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid or urine. More preferably, the sample is a blood, serum or plasma sample obtained from a patient or a blood supply. The polypeptide(s) are used in an assay, as described below, to determine the presence or absence of antibodies to the polypeptide(s) in the sample, relative to a predetermined cut-off value. The presence of such antibodies indicates the presence of mycobacterial infection.

In embodiments in which more than one polypeptide is employed, the polypeptides used are preferably complementary (*i.e.*, one component polypeptide will tend to detect infection in samples where the infection would not be detected by another component polypeptide). Complementary polypeptides may generally be identified by using each polypeptide individually to evaluate serum samples obtained from a series of patients known to be infected with a *Mycobacterium*. After determining which samples test positive (as described below) with each polypeptide, combinations of two or more polypeptides may be formulated that are capable of detecting infection in most, or all, of the samples tested. For example, approximately 25-30% of sera from tuberculosis-infected individuals are negative for antibodies to any single protein, such as the 38 kDa antigen mentioned above. Complementary polypeptides may, therefore, be used in combination with the 38 kDa antigen to improve sensitivity of a diagnostic test.

A variety of assay formats employing one or more polypeptides to detect antibodies in a sample are well known in the art. *See, e.g., Harlow and Lane, Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In a preferred embodiment, the assay involves the use of polypeptide immobilized on a solid support to bind to and remove the antibody from the sample. The bound antibody may then be detected using a detection reagent that contains a reporter group. Suitable detection reagents include antibodies that bind to the antibody/polypeptide complex and free polypeptide labelled with a reporter group (*e.g., in a semi-competitive assay*). Alternatively, a competitive assay may be utilized, in which an antibody that binds to the polypeptide is labelled with a reporter group and allowed to bind to the immobilized antigen after incubation of the antigen with the sample. The extent to which components of the sample inhibit the binding of the labelled antibody to the polypeptide is indicative of the reactivity of the sample with the immobilized polypeptide.

The solid support may be any solid material to which the antigen may be attached. Suitable materials are well known in the art. For example, the solid support may be a test well in a microtiter plate or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681.

The polypeptides may be bound to the solid support using a variety of techniques well known in the art. In the context of the present invention, the term "bound" refers to both noncovalent association, such as adsorption, and covalent attachment, which may be a direct linkage between the antigen and functional groups on the support or a linkage by way of a cross-linking agent. Binding by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the polypeptide, in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and 1 day. In general, contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of polypeptide ranging from about 10 ng to about 1 μ g, and preferably about 100 ng, is sufficient to bind an adequate amount of antigen.

Covalent attachment of polypeptide to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the polypeptide. For example, the polypeptide may be bound to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the polypeptide (*see, e.g.,* Pierce Immunotechnology Catalog and Handbook, 1991, at A12-A13).

In certain embodiments, the assay is an enzyme-linked immunosorbent assay (ELISA). This assay may be performed by first contacting a polypeptide antigen that has been immobilized on a solid support, with the sample, such that antibodies to the polypeptide within the sample are allowed to bind to the immobilized polypeptide. Unbound sample is then removed from the immobilized polypeptide and a detection reagent capable of binding to the immobilized antibody-polypeptide complex is added. The amount of detection reagent that remains bound to the solid support is then determined using a method appropriate for the specific detection reagent.

More specifically, once the polypeptide is immobilized on the support as described above, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin or Tween 20™ (Sigma Chemical Co., St. Louis, MO) may be employed. The immobilized polypeptide is then incubated with the sample, and antibody is allowed to bind to the antigen. The sample may be diluted with a suitable diluent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact time, or incubation time, is that period of time that is sufficient to detect the presence of antibody within a *M. tuberculosis*-infected sample. Preferably, the contact time is sufficient to achieve a level of binding that is at least 95% of that achieved at equilibrium between bound and unbound antibody. The time necessary to achieve equilibrium may be readily determined by assaying the level of binding that occurs over a period of time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

Unbound sample may be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.1% Tween 20™. Detection reagent may then be added to the solid support. An appropriate detection reagent is any compound that binds to the immobilized antibody-polypeptide complex and that can be detected by any of a variety of means known in the art. Preferably, the detection reagent contains a binding agent (such as, for example, Protein A, Protein G, immunoglobulin, lectin or free antigen) conjugated to a reporter group. Preferred reporter groups include enzymes (such as horseradish peroxidase), substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. The conjugation of binding agent to reporter group may be achieved using standard methods known in the art. Common binding agents may also be purchased conjugated to a variety of reporter groups from many commercial sources (*e.g.*, Zymed Laboratories, San Francisco, CA, and Pierce, Rockford, IL).

The detection reagent is incubated with the immobilized antibody-polypeptide complex for an amount of time sufficient to detect the bound antibody. An appropriate amount of time may generally be determined from the manufacturer's instructions or by assaying the level of binding that occurs over a period of time. Unbound detection reagent is then removed and bound detection reagent is detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products.

To determine the presence or absence of anti-mycobacterial antibodies in the sample, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. In one preferred embodiment, the cut-off value is the average mean signal obtained when the immobilized antigen is incubated with samples from an uninfected patient. In an alternate

preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett et al., *Clinical Epidemiology: A Basic Science for Clinical Medicine*, Little Brown and Co., 1985, pp. 106-107. In general, signals higher than the predetermined cut-off value are considered to be positive for mycobacterial infection.

The assay may also be performed in a rapid flow-through or strip test format, wherein the antigen is immobilized on a membrane, such as nitrocellulose. In the flow-through test, antibodies within the sample bind to the immobilized polypeptide as the sample passes through the membrane. A detection reagent (*e.g.*, protein A-colloidal gold) then binds to the antibody-polypeptide complex as the solution containing the detection reagent flows through the membrane. The detection of bound detection reagent may then be performed as described above. In the strip test format, one end of the membrane to which polypeptide is bound is immersed in a solution containing the sample. The sample migrates along the membrane through a region containing detection reagent and to the area of immobilized polypeptide. Concentration of detection reagent at the polypeptide indicates the presence of anti-mycobacterial antibodies in the sample. Typically, the concentration of detection reagent at that site generates a pattern, such as a line, that can be read visually. The absence of such a pattern indicates a negative result. In general, the amount of polypeptide immobilized on the membrane is selected to generate a visually discernible pattern when the biological sample contains a level of antibodies that would be sufficient to generate a positive signal in an ELISA, as discussed above. Preferably, the amount of polypeptide immobilized on the membrane ranges from about 25 ng to about 1 μ g, and more preferably from about 50 ng to about 500 ng. Such tests can typically be performed with a very small amount (*e.g.*, one drop) of patient serum or blood.

Numerous other assay protocols exist that are suitable for use with the polypeptides of the present invention. The above descriptions are intended to be exemplary only.

The present invention also provides antibodies to the inventive polypeptides. Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art. See, *e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In one such technique, an immunogen comprising the antigenic

polypeptide is initially injected into any of a wide variety of mammals (*e.g.*, mice, rats, rabbits, sheep and goats). The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for the antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.* 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e.*, reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells may then be immortalized by fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal, using one of a variety of techniques well known in the art.

Monoclonal antibodies may be isolated from the supernatants of the resulting hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood.

Antibodies may be used in diagnostic tests to detect the presence of mycobacterial antigens using assays similar to those detailed above and other techniques well known to those of skill in the art, thereby providing a method for detecting mycobacterial infection, such as *M. tuberculosis* infection, in a patient.

Diagnostic reagents of the present invention may also comprise polynucleotides encoding one or more of the above polypeptides, or one or more portions thereof. For example, primers comprising at least 10 contiguous oligonucleotides of an inventive polynucleotide may be used in polymerase chain reaction (PCR) based tests. Similarly, probes comprising at least 18 contiguous oligonucleotides of an inventive polynucleotide may

be used for hybridizing to specific sequences. Techniques for both PCR based tests and hybridization tests are well known in the art. Primers or probes may thus be used to detect *M. tuberculosis* and other mycobacterial infections in biological samples, preferably sputum, blood, serum, saliva, cerebrospinal fluid or urine. DNA probes or primers comprising oligonucleotide sequences described above may be used alone, in combination with each other, or with previously identified sequences, such as the 38 kDa antigen discussed above.

The word "about," when used in this application with reference to a percentage by weight composition, contemplates a variance of up to 10 percentage units from the stated percentage. When used in reference to percentage identity or percentage probability, the word "about" contemplates a variance of up to one percentage unit from the stated percentage.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLE 1

EFFECT OF IMMUNIZATION OF MICE WITH *M. VACCAE* ON TUBERCULOSIS

This example illustrates the effect of immunization with heat-killed *M. vaccae* or *M. vaccae* culture filtrate in mice prior to challenge with live *M. tuberculosis*.

M. vaccae (ATCC Number 15483) was cultured in sterile Medium 90 (yeast extract, 2.5 g/l; tryptone, 5 g/l; glucose, 1 g/l) at 37 °C. The cells were harvested by centrifugation, and transferred into sterile Middlebrook 7H9 medium (Difco Laboratories, Detroit, MI, USA) with glucose at 37 °C for one day. The medium was then centrifuged to pellet the bacteria, and the culture filtrate removed. The bacterial pellet was resuspended in phosphate buffered saline at a concentration of 10 mg/ml, equivalent to 10^{10} *M. vaccae* organisms per ml. The cell suspension was then autoclaved for 15 min at 120 °C. The culture filtrate was passaged through a 0.45 µm filter into sterile bottles.

As shown in Fig.1A, when mice were immunized with 1 mg, 100 µg or 10 µg of *M. vaccae* and infected three weeks later with 5×10^5 colony forming units (CFU) of live *M. tuberculosis* H37Rv, significant protection from infection was seen. In this example, spleen,

liver and lung tissue was harvested from mice three weeks after infection, and live bacilli determined (expressed as CFU). The reduction in bacilli numbers, when compared to tissue from non-immunized control mice, exceeded 2 logs in liver and lung tissue, and 1 log in spleen tissue. Immunization of mice with heat-killed *M. tuberculosis* H37Rv had no significant protective effects on mice subsequently infected with live *M. tuberculosis* H37Rv.

Fig.1B shows that when mice were immunized with 100 µg of *M. vaccae* culture filtrate, and infected three weeks later with 5×10^5 CFU of *M. tuberculosis* H37Rv, significant protection was also seen. When spleen, liver and lung tissue was harvested from mice three weeks after infection, and live bacilli numbers (CFU) determined, a 1-2 log reduction in numbers, as compared to non-immunized control mice, was observed.

EXAMPLE 2

EFFECT OF INTRADERMAL AND INTRA-LUNG ROUTES OF IMMUNISATION WITH *M. VACCAE* ON TUBERCULOSIS IN CYNOMOLGOUS MONKEYS

This example illustrates the effect of immunisation with heat-killed *M. vaccae* or *M. vaccae* culture filtrate through intradermal and intralung routes in cynomolgous monkeys prior to challenge with live *M. tuberculosis*.

Heat-killed *M. vaccae* and *M. vaccae* culture filtrate were prepared as described above in Example 1. Five groups of cynomolgous monkeys were used, with each group containing 2 monkeys. Two groups of monkeys were immunised with whole heat-killed *M. vaccae* either intradermally or intralung; two groups of monkeys were immunised with *M. vaccae* culture filtrate either intradermally or intralung; and a control group received no immunisations. All immunogens were dissolved in phosphate buffered saline. The composition employed for immunisation, amount of immunogen, and route of administration for each group of monkeys are provided in Table 1. Prior to immunisation, all monkeys were weighed (Wt kg), body temperature was measured (temp), and a blood sample taken for determination of erythrocyte sedimentation rate (ESR mm/hr) and lymphocyte proliferation (LPA) to an *in vitro* challenge

with purified protein (PPD) prepared from *Mycobacterium bovis*. Both ESR and LPA have been used as indicators of inflammatory T cell responses. At day 33 post-immunisation these measurements were repeated. At day 34, all monkeys received a second immunisation using the same amount of *M. vaccae* and route of immunisation as the initial immunisation. On day 62, body weight, temperature, ESR and LPA to PPD were measured, then all monkeys were infected with 10^3 colony forming units of the Erdman strain of *Mycobacterium tuberculosis* by inserting the organisms directly in the right lungs of immunised animals. Twenty eight days following infection, body weight, temperature, ESR and LPA to PPD were measured in all monkeys, and the lungs were x-rayed to determine whether infection with live *M. tuberculosis* had resulted in the onset of pneumonia.

TABLE 1
COMPARISON OF INTRADERMAL AND INTRALUNG
ROUTES OF IMMUNISATION

Group Number	Identification Number of Monkey	Amount of Immunogen	Route of Immunisation
1	S3101-E	0	-
(Controls)	3144-B	0	-
2	4080-B	500 µg	intradermal
(Immunised with heat-killed <i>M. vaccae</i>)	3586-B	500 µg	intradermal
3	3534-C	500 µg	intralung
(Immunised with heat-killed <i>M. vaccae</i>)	3160-A	500 µg	intralung
4	3564-B	100 µg	intradermal
(Immunised with culture filtrate)	3815-B	100 µg	intradermal
5	4425-A	100 µg	intralung
(Immunised with culture filtrate)	2779-D	100 µg	intralung

The results of these studies are provided below in Tables 2A-E and are summarized below:

Table 2A – Twenty-eight days after infection with *M. tuberculosis* Erdman, chest x-rays of control (non-immunised) monkeys revealed haziness over the right suprahilar regions of both animals, indicating the onset of pneumonia. This progressed and by day 56 post-infection x-rays indicated disease in both lungs. As expected, as disease progressed both control animals lost weight and showed significant LPA responses to PPD, indicating strong T cell reactivity to *M. tuberculosis*. The ESR measurements were variable but consistent with strong immune reactivity.

Table 2B – The two monkeys immunised twice with 500 µg *M. vaccae* intradermally showed no sign of lung disease 84 days post-infection with *M. tuberculosis*. The LPA responses to PPD indicated there was immune reactivity to *M. tuberculosis*, and both animals continued to gain weight, a consistent indication of a lack of disease.

Table 2C – The two monkeys immunised twice with 500 µg *M. vaccae* intralung showed almost identical results to those animals of Table 2B. There was no sign of lung disease 84 days post infection with *M. tuberculosis*, with consistent weight gains. Both animals showed LPA response to PPD in the immunisation phase (day 0-62) and post-infection, indicating strong T cell reactivity had developed as a result of using the lung as the route of immunisation and subsequent infection.

Immunisation twice with 500 µg of whole *M. vaccae* has consistently shown protective effects against subsequent infection with live *M. tuberculosis*. The data presented in Tables 2D and 2E show the effects of immunisation with 100 µg of *M. vaccae* culture filtrate. Monkeys immunised intradermally showed signs of developing disease 84 days post-infection, while in those immunised intralung, one animal showed disease after 56 days and one animal showed disease 84 days post-infection. This was a significant delay in disease onset indicating that the immunisation process had resulted in some protective immunity.

TABLE 2A
CONTROL MONKEYS

ID#	Days	Wt.Kgs	Temp.	ESR Mm/hr	LPA PPD10	LPA PPD1	X-Ray Remarks
S3101E	0	2.17	37.0	0	0.47	1.1	Negative
	34	1.88	37.3	ND	0.85	1.4	ND
	62	2.02	36.0	ND	1.3	1.5	ND
→ Time of Infection							
	28	2.09	38.0	2	1.3	3.7	Positive
	56	1.92	37.2	20	5.6	9.1	Positive
	84	1.81	37.5	8	4.7	5.6	Positive
	121	DIED					

ID#	Days	Wt.Kgs	Temp.	ESR Mm/hr	LPA PPD 10µg	LPA PPD 1µg	X-Ray Remarks
3144-B	0	2.05	36.7	0	0.87	1.8	Negative
	34	1.86	37.6	ND	2.2	1.4	ND
	62	1.87	36.5	ND	1.6	1.6	ND
→ Time of Infection							
	28	2.10	38.0	0	12	8.7	Positive
	56	1.96	37.6	0	29.6	21.1	Positive
	84	1.82	37.3	4	45.3	23.4	Positive
	131	DIED					

ND = Not Done

TABLE 2B

**MONKEYS IMMUNISED
WITH WHOLE HEAT-KILLED *M. VACCAR* (500 µg)
INTRADERMAL**

ID#	Days	Wt.Kgs	Temp.	ESR Mm/hr	LPA PPD 10µg	LPA PPD 1µg	X-Ray Remarks
4080-B	0	2.05	37.1	1	1.1	0.77	Negative
	34	1.97	38.0	ND	1.7	1.4	ND
	62	2.09	36.7	ND	1.5	1.5	ND
→ Time of Infection							
	28	2.15	37.6	0	2.6	2.1	Negative
	56	2.17	37.6	0	8.2	7.6	Negative
	84	2.25	37.3	0	3.8	2.8	Negative
	178	DIED					

ID#	Days	Wt.Kgs	Temp.	ESR mm/hr	LPA PPD 10µg	LPA PPD 1µg	X-Ray Remarks
3586-B	0	2.29	37.0	0	1.1	1.4	Negative
	34	2.22	38.0	ND	1.9	1.6	ND
	62	2.39	36.0	ND	1.3	1.6	ND
→ Time of Infection							
	28	2.31	38.2	0	3.2	2.6	Negative
	56	2.32	37.2	0	7.8	4.2	Negative
	84	2.81	37.4	0	3.4	1.8	Negative
	197	DIED					

ND = Not Done

TABLE 2C

**MONKEYS IMMUNISED
WITH WHOLE HEAT-KILLED *M. VACCÆ* (500 µg)
INTRALUNG**

ID#	Days	Wt.Kgs	Temp.	ESR mm/hr	LPA PPD 10µg	LPA PPD 1µg	X-Ray Remarks
3534-C	0	2.15	36.8	0	1.7	1.3	Negative
	34	2.00	37.8	ND	4.4	1.4	ND
	62	2.13	36.4	ND	3.2	1.9	ND
→ Time of Infection							
	28	2.38	37.7	0	1.9	2.6	Negative
	56	2.42	37.8	0	5.3	4.7	Negative
	84	2.46	37.1	1	3.1	3.2	Negative
	210	No sign of lung disease					Negative

ID#	Days	Wt.Kgs	Temp.	ESR mm/hr	LPA PPD 10µg	LPA PPD 1µg	X-Ray Remarks
3160-A	0	2.17	37.3	0	1.2	0.79	Negative
	34	1.98	37.1	ND	3.9	7.8	ND
	62	2.17	36.9	ND	1.7	2.4	ND
→ Time of Infection							
	28	2.38	37.7	0	1.9	2.6	Negative
	56	2.42	37.8	0	5.3	4.7	Negative
	84	2.46	37.1	1	3.1	3.2	Negative
	210	Stable lung disease					Positive

ND = Not Done

TABLE 2D

**MONKEYS IMMUNISED
WITH CULTURE FILTRATE (100 µg)
INTRADERMAL**

ID#	Days	Wt.Kgs	Temp.	ESR mm/hr	LPA PPD 10µg	LPA PPD 1µg	X-Ray Remarks
3564-B	0	2.40	37.2	0	1.4	1.4	Negative
	34	2.42	38.1	ND	3.3	2.7	ND
	62	2.31	37.1	ND	3.1	3.4	ND
→ Time of Infection							
	28	2.41	38.6	13	24	13.6	Negative
	56	2.38	38.6	0	12.7	12.0	Negative
	84	2.41	38.6	2	21.1	11.8	Positive
	140						Died

ID#	Days	Wt.Kgs	Temp.	ESR mm/hr	LPA PPD 10µg	LPA PPD 1µg	X-Ray Remarks
3815-B	0	2.31	36.3	0	1.0	1.4	Negative
	34	2.36	38.2	ND	1.9	2.0	ND
	62	2.36	36.4	ND	3.7	2.8	ND
→ Time of Infection							
	28	2.45	37.8	0	2.1	3.3	Negative
	56	2.28	37.3	4	8.0	5.6	Negative
	84	2.32	37.4	0	1.9	2.2	Positive
	210						Positive

ND = Not Done

TABLE 2E

**MONKEYS IMMUNISED
WITH CULTURE FILTRATE (100 µg)
INTRALUNG**

ID#	Days	Wt.Kgs	Temp.	ESR mm/hr	LPA PPD 10µg	LPA PPD 1µg	X-Ray Remarks
4425-A	0	2.05	36.0	0	0.35	1.2	Negative
	34	2.0	37.6	ND	3.0	2.4	ND
	62	2.11	37.6	ND	2.2	1.6	ND
→ Time of Infection							
	28	2.21	38.0	0	8.4	4.1	Negative
	56	2.11	37.6	0	23.9	17.7	Negative
	84	2.18	37.9	0	8.4	7.2	Positive
	210	Stable lung disease					Positive

ID#	Days	Wt.Kgs	Temp.	ESR mm/hr	LPA PPD 10µg	LPA PPD 1µg	X-Ray Remarks
2779-D	0	2.56	38.6	2	1.9	1.4	Negative
	28	2.55	37.9	ND	0.78	1.1	ND
	56	2.69	38.4	ND	1.3	1.5	ND
→ Time of Infection							
	56	2.25	39.0	24	ND	ND	Positive
	96						Died

ND = Not Done

EXAMPLE 3
EFFECT OF IMMUNISATION WITH *M. VACCAE*
ON ASTHMA IN MICE

This example demonstrates that both heat-killed *M. vaccae* and DD-*M. vaccae*, when administered to mice via the intranasal route, are able to inhibit the development of an allergic immune response in the lungs. This was demonstrated in a mouse model of the asthma-like allergen specific lung disease. The severity of this allergic disease is reflected in the large numbers of eosinophils that accumulate in the lungs.

C57BL/6J mice were given 2 µg ovalbumin in 100 µl alum adjuvant by the intraperitoneal route at time 0 and 14 days, and subsequently given 100 µg ovalbumin in 50 µl phosphate buffered saline (PBS) by the intranasal route on day 28. The mice accumulated eosinophils in their lungs as detected by washing the airways of the anaesthetised mice with saline, collecting the washings (broncheolar lavage or BAL), and counting the numbers of eosinophils.

As shown in Figs. 2A and B, groups of seven mice administered either 10 or 1000 µg of heat-killed *M. vaccae* (Fig. 2A), or 10, 100 or 200 µg of DD-*M. vaccae*, prepared as described below (Fig. 2B) intranasally 4 weeks before intranasal challenge with ovalbumin, had reduced percentages of eosinophils in the BAL cells collected 5 days after challenge with ovalbumin compared to control mice. Control mice were given intranasal PBS. Live *M. bovis* BCG at a dose of 2×10^5 colony forming units also reduced lung eosinophilia. The data in Figs. 2A and B show the mean and SEM per group of mice.

Figs. 2C and D show that mice given either 1000 µg of heat-killed *M. vaccae* (Fig. 2C) or 200 µg of DD-*M. vaccae* (Fig. 2D) intranasally as late as one week before challenge with ovalbumin had reduced percentages of eosinophils compared to control mice. In contrast, treatment with live BCG one week before challenge with ovalbumin did not inhibit the development of lung eosinophilia when compared with control mice.

As shown in Fig. 2E, immunisation with either 1 mg of heat-killed *M. vaccae* or 200 µg of DD-*M. vaccae*, given either intranasally (i.n.) or subcutaneously (s.c.), reduced lung

eosinophilia following challenge with ovalbumin when compared to control animals given PBS. In the same experiment, immunization with BCG of the Pasteur (BCG-P) and Connought (BCG-C) strains prior to challenge with ovalbumin also reduced the percentage of eosinophils in the BAL of mice.

Eosinophils are blood cells that are prominent in the airways in allergic asthma. The secreted products of eosinophils contribute to the swelling and inflammation of the mucosal linings of the airways in allergic asthma. The data shown in Figs. 2A-E indicate that treatment with heat-killed *M. vaccae* or DD-*M. vaccae* reduces the accumulation of lung eosinophils, and may be useful in reducing inflammation associated with eosinophilia in the airways, nasal mucosal and upper respiratory tract.

DD-*M.vaccae* depleted of mycolic acids and arabinogalactan

Mycolic acids were depleted from DD-*M.vaccae* by treatment with potassium hydroxide (0.5% KOH) in ethanol for 48 hours at 37°C. Mycolic acid depleted DD-*M.vaccae* cells were then washed with ethanol and ether and dried. Arabinogalactans were depleted from the KOH treated DD-*M.vaccae* by further treatment with 1% periodic acid in 3% acetic acid for 1 hr at room temperature followed by treatment with sodium borohydride 0.1M for 1 hour at room temperature. After arabinogalactan depletion, samples were washed with water and lyophilized. As shown in Table 3, both mycolate depleted DD-*M.vaccae* as well as mycolic acid and arabinogalactan depleted DD-*M.vaccae*, given intranasally to ovalbumin sensitized mice reduced the accumulation of eosinophils in the bronchoalveolar lavage fluid following challenge with ovalbumin.

Administration of heat-killed *M. vaccae*, DD-*M. vaccae* or DD-*M.vaccae* depleted of mycolic acids and arabinogalactan may therefore reduce the severity of asthma and diseases that involve similar immune abnormalities, such as allergic rhinitis.

In addition, serum samples were collected from mice in the experiment shown in Fig. 2E and antibodies to ovalbumin was measured by standard enzyme-linked immunoassay (EIA). As shown in Table 3A below, sera from mice infected with BCG had higher levels of ovalbumin specific IgG1 than sera from PBS controls. In contrast, mice

immunized with *M. vaccae* or DD-*M. vaccae* had similar or lower levels of ovalbumin-specific IgG1. As IgG1 antibodies are characteristic of a Th2 immune response, these results are consistent with the suppressive effects of heat-killed *M. vaccae* and DD-*M. vaccae* on the asthma-inducing Th2 immune responses.

TABLE 3

DECREASED LUNG EOSINOPHILIA IN MICE TREATED WITH MYCOLIC ACID
DEPLETED DD-*M. VACCAE* OR MYCOLIC ACID AND ARABINOGALACTAN
DEPLETED DD-*M. VACCAE*.

Treatment Group	% Eosinophils in BAL	
	Mean	S.E.M.
PBS	58.8	8.4
Mycolic acid depleted DD- <i>M. vaccae</i>	21.8	17.4
Mycolic acid and arabinogalactan depleted DD- <i>M. vaccae</i>	16.8	0.3

Note: At least 7 mice per group.

TABLE 3A

LOW ANTIGEN-SPECIFIC IgG1 SERUM LEVELS
IN MICE IMMUNIZED WITH HEAT-KILLED *M. VACCAE* OR DD-*M. VACCAE*

Treatment Group	Serum IgG1	
	Mean	SEM
<i>M. vaccae</i> i.n.	185.00	8.3
<i>M. vaccae</i> s.c.	113.64	8.0
DD- <i>M. vaccae</i> i.n.	96.00	8.1
DD- <i>M. vaccae</i> s.c.	110.00	4.1
BCG, Pasteur	337.00	27.2
BCG, Connaught	248.00	46.1
PBS	177.14	11.4

Note: Ovalbumin-specific IgG1 was detected using anti-mouse IgG1 (Serotec). Group means are expressed as the reciprocal of the EU50 end point titre.

EXAMPLE 4

EFFECT OF IMMUNIZING MICE WITH *M. VACCAE*, DD-*M. VACCAE* OR RECOMBINANT *M. VACCAE* PROTEINS ON TUBERCULOSIS

This example illustrates the effect of immunization with heat-killed *M.vaccae*, DD-*M.vaccae* or recombinant *M. vaccae* proteins without additional adjuvants, or a combination of heat-killed *M.vaccae* with a pool of recombinant proteins derived from *M.vaccae*.

Mice were injected intraperitoneally with one of the following preparations on two occasions three weeks apart:

- a) Phosphate buffered saline (PBS, control);
- b) Heat-killed *M.vaccae* (500 ug);
- c) DD-*M.vaccae* (50 ug);
- d) A pool of recombinant proteins containing 15 ug of each of GV4P, GV7, GV9, GV27B, GV33 protein (prepared as described below); and
- e) Heat-killed *M.vaccae* plus the pool of recombinant proteins

Three weeks after the last intraperitoneal immunization, the mice were infected with 5×10^5 live H37Rv *M.tuberculosis* organisms. After a further three weeks, the mice were sacrificed, and their spleens homogenized and assayed for colony forming units (CFU) of *M.tuberculosis* as an indicator of severity of infection.

Figs. 3A and 3B show data in which each point represents individual mice. The numbers of CFU recovered from control mice immunised with PBS alone were taken as the baseline. All data from experimental mice were expressed as number of logarithms of CFUs below the baseline for control mice (or log protection). As shown in Fig. 3A, mice immunized with heat-killed *M.vaccae* or DD-*M.vaccae* showed a mean reduction of >1 or 0.5 logs CFU, respectively.

As shown in Fig. 3B, the spleens of mice immunized with the pool of recombinant proteins containing GV4P, GV7, GV9, GV27B and GV33, had CFUs slightly less than control mice. However, when GV4P, GV7, GV9, GV27B and GV33 were given in combination with heat-killed *M.vaccae*, the reduction in CFUs exceeded a mean of >1.5 logs.

The data demonstrates the effectiveness of immunization with *M.vaccae*, DD-*M.vaccae* or recombinant proteins derived from *M.vaccae* against subsequent infection with tuberculosis, and further indicates that *M.vaccae*, DD-*M.vaccae* and recombinant proteins may be developed as vaccines against tuberculosis.

EXAMPLE 5

EFFECT OF INTRADERMAL INJECTION OF HEAT-KILLED MYCOBACTERIUM VACCAE ON PSORIASIS IN HUMAN PATIENTS

This example illustrates the effect of two intradermal injections of heat-killed *Mycobacterium vaccae* on psoriasis in human patients.

M. vaccae (ATCC Number 15483) was cultured in sterile Medium 90 (yeast extract, 2.5g/l; tryptone, 5g/l; glucose, 1 g/l) at 37 °C. The cells were harvested by centrifugation, and transferred into sterile Middlebrook 7H9 medium (Difco Laboratories, Detroit, MI, USA) with glucose at 37 °C for one day. The medium was then centrifuged to pellet the bacteria, and the culture filtrate removed. The bacterial pellet was resuspended in phosphate buffered saline at a concentration of 10 mg/ml, equivalent to 10^{10} *M. vaccae* organisms per ml. The cell suspension was then autoclaved for 15 min at 120 °C and stored frozen at -20 °C. Prior to use the *M. vaccae* suspension was thawed, diluted to a concentration of 5 mg/ml in phosphate buffered saline, autoclaved for 15 min at 120 °C and 0.2 ml aliquoted under sterile conditions into vials for use in patients.

Twenty-four volunteer psoriatic patients, male and female, 15-61 years old with no other systemic diseases were admitted to treatment. Pregnant patients were not included. The patients had PASI scores of 12-35. The PASI score is a measure of the location, size and degree of skin scaling in psoriatic lesions on the body. A PASI score of above 12 reflects

widespread disease lesions on the body. The study commenced with a washout period of four weeks where the patients did not have systemic anti-psoriasis treatment or effective topical therapy.

The 24 patients were then injected intradermally with 0.1 ml *M. vaccae* (equivalent to 500 µg). This was followed three weeks later with a second intradermal injection with the same dose of *M. vaccae* (500 µg). Psoriasis was evaluated from four weeks before the first injection of heat-killed *M. vaccae* to twelve weeks after the first injection as follows:

- A. The PASI scores were determined at -4, 0, 3, 6 and 12 weeks;
- B. Patient questionnaires were completed at 0, 3, 6 and 12 weeks; and
- C. Psoriatic lesions and each patient were photographed at 0, 3, 6, 9 and 12 weeks.

The data shown in Table 4 describe the age, sex and clinical background of each patient.

TABLE 4
Patient Data in the Study of the Effect of *M. vaccae* in Psoriasis

Code No.	Patient	Age/Sex	Duration of Disorder	Admission PASI Score
PS-001	D.C.	49/F	30 years	28.8
PS-002	E.S.	41/F	4 months	19.2
PS-003	M.G.	24/F	8 months	18.5
PS-004	D.B.	54/M	2 years	12.2
PS-005	C.E.	58/F	3 months	30.5
PS-006	M.G.	18/F	3 years	15.0
PS-007	L.M.	27/M	3 years	19.0
PS-008	C.C	21/F	1 month	12.2
PS-009	E.G	42/F	5 months	12.6
PS-010	J.G	28/M	7 years	19.4
PS-011	J.U	39/M	1 year	15.5
PS-012	C.S	47/M	3 years	30.9
PS-013	H.B	44/M	10 years	30.4
PS-014	N.J	41/M	17 years	26.7
PS-015	J.T	61/F	15 years	19.5
PS-016	L.P	44/M	5 years	30.2
PS-017	E.N	45/M	5 years	19.5
PS-018	E.L	28/F	19 years	16.0
PS-019	B.A	38/M	17 years	12.3
PS-020	P.P	58/F	1 year	13.6
PS-021	L.I	27/F	8 months	22.0
PS-022	A.C	20/F	7 months	26.5
PS-023	C.A	61/F	10 years	12.6
PS-024	F.T	39/M	15 years	29.5

All patients demonstrated a non-ulcerated, localised erythematous soft indurated reaction at the injection site. No side effects were noted, or complained of by the patients. The data shown in Table 5, below, are the measured skin reactions at the injection site, 48 hours, 72 hours and 7 days after the first and second injections of heat-killed *M. vaccae*. The data shown in Table 6, below, are the PASI scores of the patients at the time of the first injection of *M. vaccae* (Day 0) and 3, 6, 9, 12 and 24 weeks later.

It can clearly be seen that, by week 9 after the first injection of *M. vaccae*, 16 of 24 patients showed a significant improvement in PASI scores. Seven of fourteen patients who have completed 24 weeks of follow-up remained stable with no clinical sign of redevelopment of severe disease. These results demonstrate the effectiveness of multiple intradermal injections of inactivated *M. vaccae* in the treatment of psoriasis. PASI scores below 10 reflect widespread healing of lesions. Histopathology of skin biopsies indicated that normal skin structure is being restored. Only one of the first seven patients who have completed 28 weeks follow-up has had a relapse.

TABLE 5
Skin Reaction Measurements in Millimeter

Code No.	Time of Measurement					
	First Injection			Second Injection		
	48 hours	72 hours	7 days	48 hours	72 hours	7 days
PS-001	12x10	12x10	10x8	15x14	15x14	10x10
PS-002	18x14	20x18	18x14	16x12	18x12	15x10
PS-003	10x10	14x10	10x8	15x12	15x10	10x10
PS-004	14x12	22x18	20x15	20x20	20x18	14x10
PS-005	10x10	13x10	DNR	DNR	DNR	DNR
PS-006	10x8	10x10	6x4	12x10	15x15	10x6
PS-007	15x15	18x16	12x10	15x13	15x12	12x10
PS-008	18x18	13x12	12x10	18x17	15x10	15x10
PS-009	13x13	18x15	12x8	15x13	12x12	12x7
PS-010	13x11	15x15	8x8	12x12	12x12	5x5
PS-011	17x13	14x12	12x11	12x10	12x10	12x10
PS-012	17x12	15x12	9x9	10x10	10x6	8x6
PS-013	18x11	15x11	15x10	15x10	15x13	14x6
PS-014	15x12	15x11	15x10	13x12	14x10	8x5
PS-015	15x12	16x12	15x10	7x6	14x12	6x4
PS-016	6x5	6x6	6x5	8x8	9x8	9x6
PS-017	20x15	15x14	14x10	15x15	17x16	DNR
PS-018	14x10	10x8	10x8	12x12	10x10	10x10
PS-019	10x10	14x12	10x8	DNR	15x14	15x14
PS-020	15x12	15x15	12x15	15x15	14x12	13x12
PS-021	15x12	15x12	7x4	11x10	11x10	11x8
PS-022	12x10	10x8	10x8	15x12	13x10	10x8
PS-023	13x12	14x12	10x10	17x17	15x15	DNR

Code No.	Time of Measurement					
	10x10	10x10	10x8	10x8	8x7	8x7
PS-024						

DNR = Did not report.

TABLE 6

Clinical Status of Patients after Injection of *M. vaccae* (PASI Scores)

Code No.	Day 0	Week 3	Week 6	Week 9	Week 12	Week 24
PS-001	28.8	14.5	10.7	2.2	0.7	0
PS-002	19.2	14.6	13.6	10.9	6.2	0.6
PS-003	18.5	17.2	10.5	2.7	1.6	0
PS-004	12.2	13.4	12.7	7.0	1.8	0.2
PS-005*	30.5	DNR	18.7	DNR	DNR	0
PS-006	15.0	16.8	16.4	2.7	2.1	3.0
PS-007	19.0	15.7	11.6	5.6	2.2	0
PS-008	12.2	11.6	11.2	11.2	5.6	0
PS-009	12.6	13.4	13.9	14.4	15.3	13.0
PS-010	18.2	16.0	19.4	17.2	16.9	19.3
PS-011	17.2	16.9	16.7	16.5	16.5	15.5
PS-012	30.9	36.4	29.7	39.8**		
PS-013	19.5	19.2	18.9	17.8	14.7	17.8
PS-014	26.7	14.7	7.4	5.8	9.9	24.4***
PS-015	30.4	29.5	28.6	28.5	28.2	24.3
PS-016	30.2	16.8	5.7	3.2	0.8	3.3
PS-017	12.3	12.6	12.6	12.6	8.2	8.7
PS-018	16.0	13.6	13.4	13.4	13.2	12.8
PS-019	19.5	11.6	7.0	DNR	DNR	DNR
PS-020	13.6	13.5	12.4	12.7	12.4	4.4

PS-021	22.0	20.2	11.8	11.4	15.5	15.7
PS-022	26.5	25.8	20.7	11.1	8.3	5.6
PS-023	12.6	9.2	6.6	5.0	4.8	12.6
PS-024	29.5	27.5	20.9	19.0	29.8	21.2

- * Patient PS-005 received only one dose of autoclaved *M.vaccae*.
- ** Patient PS-012 removed from trial, drug (penicillin) induced dermatitis
- *** Patient PS-014 was revaccinated
- DNR = Did not report

Patients treated with *M.vaccae* may achieve remission (PASI score = 0). The remission or improvement of PASI score may be long lasting. By example, Patient PS-003 achieved remission by week 20 and was still in remission at week 80. Overall 13 of 24 patients showed a greater than 50% improvement in PASI scores.

Patient PS-001 achieved remission at week 16, relapsed at week 48 (PASI 2.7), was re-vaccinated with injections of *M.vaccae* and subsequently improved with PASI falling from 17.8 (Week 60) to 0.8 (week 84). Thus patients may benefit from repeated treatment.

EXAMPLE 6

EFFECT OF INTRADERMAL INJECTION OF DD-*M.VACCAE* ON PSORIASIS IN HUMAN PATIENTS

This example illustrates the effect of two intradermal injections of DD-*M. vaccae* on psoriasis.

Seven volunteer psoriatic patients, male and female, 18-45 years old with no other systemic diseases were admitted to treatment. Pregnant patients were not included. The patients had PASI scores of 12-24. As discussed above, the PASI score is a measure of the location, size and degree of skin scaling in psoriatic lesions on the body. A PASI score of

above 12 reflects widespread disease lesions on the body. The study commenced with a washout period of four weeks where the four patients did not have systemic antipsoriasis treatment or effective topical therapy. The seven patients were then injected intradermally with 0.1 ml DD-*M. vaccae* (equivalent to 100 µg). This was followed three weeks later with a second intradermal injection with the same dose of DD-*M. vaccae* (100 µg).

Psoriasis was evaluated from four weeks before the first injection of *M. vaccae* to six weeks after the first injection as follows:

- A. the PASI scores were determined at -4, 0, 3 and 6 weeks;
- B. patient questionnaires were completed at 0, 3 and 6 weeks; and
- C. psoriatic lesions and each patient were photographed at 0 and 3 weeks.

The data shown in Table 7 describe the age, sex and clinical background of each patient.

TABLE 7

Patient Data in the Study of the Effect of DD-*M. vaccae* in Psoriasis

Code No.	Patient	Age/Sex	Duration of Disorder	Admission PASI Score
PS-025	A.S	25/F	2 years	12.2
PS-026	M.B	45/F	3 months	14.4
PS-027	A.G	34/M	14 years	24.8
PS-028	E.M	31/M	4 years	18.2
PS-029	A.L	44/M	5 months	18.6
PS-030	V.B	42/M	5years	21.3
PS-031	R.A	18/M	3 months	13.0

All patients demonstrated a non-ulcerated, localised erythematous soft indurated reaction at the injection site. No side effects were noted, or complained of by the patients. The data shown in Table 8 are the measured skin reactions at the injection site, 48 hours, 72 hours and 7 days after the first injection of DD-*M. vaccae*, and 48 hours and 72 hours after the second injection.

TABLE 8
Skin Reaction Measurements in Millimeters

Code No.	Time of Measurement				
	First Injection			Second Injection	
	48 hours	72 hours	7 days	48 hours	72 hours
PS-025	8x8	8x8	3x2	10x10	10x10
PS-026	12x12	12x12	8x8	DNR	14x14
PS-027	9x8	10x10	10x8	9x5	9x8
PS-028	10x10	10x10	10x8	10x10	10x10
PS-029	8x6	8x6	5x5	8x8	8x8
PS-030	14x12	14x14	10x10	12x10	12x10
PS-031	10x10	12x12	10x6	14x12	12x10

DNR = Did not report

The data shown in Table 9 are the PASI scores of the seven patients at the time of the first injection of DD-*M. vaccae* (Day 0), 3, 6, 12 and 24 weeks later.

TABLE 9
Clinical Status of Patients after Injection of DD-*M. vaccae* (PASI Scores)

Code No.	Day 0	Week 3	Week 6	Week 12	Week 24
PS-025	12.2	4.1	1.8	1.4	1.7
PS-026	14.4	11.8	6.0	6.9	1.4
PS-027	24.8	23.3	18.3	9.1	10.6
PS-028	18.2	24.1	28.6	Dropped	
PS-029	18.6	9.9	7.4	3.6	0.8
PS-030	21.3	15.7	13.9	16.5	13.6
PS-031	13.0	5.1	2.1	1.6	0.3

It can clearly be seen that by week 3 after the first injection of DD-*M. vaccae*, five patients showed a significant improvement in PASI scores. By week 24, six of seven patients showed a significant improvement in PASI score.

By way of example, Patient PS-031 went into remission (PASI score = 0) at week 32 and remained in remission when seen at week 48. The PASI score of patient PS-025 was reduced to less than 1 for more than 12 weeks. Upon an exacerbation of psoriasis (PASI = 15.8) at week 48, the patient was re-treated with DD-*M. vaccae* and improved promptly with PASI scores falling to 6.8 and 0.6 at weeks 52 and 56 respectively.

Thus treatment of psoriasis with DD-*M. vaccae* may lead to disease remission or provide prolonged benefit. Patients may also benefit with repeated treatment.

EXAMPLE 7

PREPARATION OF COMPOSITIONS FROM *M. VACCAE*

This example illustrates the processing of different constituents of *M. vaccae*.

Preparation of Delipidated and Deglycolipidated (DD-) *M. vaccae* and Compositional Analysis

Heat-killed *M. vaccae* was prepared as described as above in Example 1. To prepare delipidated *M. vaccae*, the autoclaved *M. vaccae* was pelleted by centrifugation, the pellet washed with water, collected again by centrifugation and then freeze-dried. An aliquot of this freeze-dried *M. vaccae* was set aside and referred to as lyophilised *M. vaccae*. When used in experiments it was resuspended in PBS to the desired concentration. Freeze-dried *M. vaccae* was treated with chloroform/methanol (2:1) for 60 mins at room temperature to extract lipids, and the extraction was repeated once. The delipidated residue from chloroform/methanol extraction was further treated with 50% ethanol to remove glycolipids by refluxing for two hours. The 50% ethanol extraction was repeated two times. The pooled 50% ethanol extracts were used as a source of *M. vaccae* glycolipids (see below). The residue from the 50% ethanol extraction was freeze-dried and weighed. The amount of delipidated and deglycolipidated *M. vaccae* prepared was equivalent to 11.1% of the starting wet weight of

M. vaccae used. For bioassay, the delipidated and deglycolipidated *M. vaccae* (DD-*M. vaccae*), was resuspended in phosphate-buffered saline by sonication, and sterilised by autoclaving.

The compositional analyses of heat-killed *M. vaccae* and DD-*M. vaccae* are presented in Table 9. Major changes are seen in the fatty acid composition and amino acid composition of DD-*M. vaccae* as compared to the insoluble fraction of heat-killed *M. vaccae*. The data presented in Table 9 show that the insoluble fraction of heat-killed *M. vaccae* contains 10% w/w of lipid, and the total amino acid content is 2750 nmoles/mg, or approximately 33% w/w. DD-*M. vaccae* contains 1.3% w/w of lipid and 4250 nmoles/mg amino acids, which is approximately 51% w/w.

TABLE 9

Compositional analyses of heat-killed *M. vaccae* and DD-*M. vaccae*

MONOSACCHARIDE COMPOSITION

sugar alditol	<i>M. vaccae</i>	DD-<i>M. vaccae</i>
Inositol	3.2%	1.7%
Ribitol *	1.7%	0.4%
Arabinitol	22.7%	27.0%
Mannitol	8.3%	3.3%
Galactitol	11.5%	12.6%
Glucitol	52.7%	55.2%

FATTY ACID COMPOSITION

Fatty acid	<i>M. vaccae</i>	DD-<i>M. vaccae</i>
C14:0	3.9%	10.0%
C16:0	21.1%	7.3%
C16:1	14.0%	3.3%
C18:0	4.0%	1.5%
C18:1*	1.2%	2.7%
C18:1w9	20.6%	3.1%
C18:1w7	12.5%	5.9%
C22:0	12.1%	43.0%
C24:1*	6.5%	22.9%

The insoluble fraction of heat-killed *M. vaccae* contains 10% w/w of lipid, and DD-*M. vaccae* contains 1.3% w/w of lipid.

AMINO ACID COMPOSITION

Nmoles/mg	<i>M. vaccae</i>	DD- <i>M. vaccae</i>
ASP	231	361
THR	170	266
SER	131	199
GLU	319	505
PRO	216	262
GLY	263	404
ALA	416	621
CYS*	24	26
VAL	172	272
MET*	72	94
ILE	104	171
LEU	209	340
TYR	39	75
PHE	76	132
GlcNH ₂	5	6
HIS	44	77
LYS	108	167
ARG	147	272

The total amino acid content of the insoluble fraction of heat-killed *M. vaccae* is 2750 nmoles/mg, or approximately 33% w/w. The total amino acid content of DD-*M. vaccae* is 4250 nmoles/mg, or approximately 51% w/w.

Comparison of composition of DD-*M. vaccae* with delipidated and deglycolipidated forms of *M. tuberculosis* and *M. smegmatis*

Delipidated and deglycolipidated *M. tuberculosis* and *M. smegmatis* were prepared using the procedure described above for delipidated and deglycolipidated *M. vaccae*. As indicated in Table 10, the profiles of the percentage composition of amino acids in DD-*M. vaccae*, DD-*M. tuberculosis* and DD-*M. smegmatis* showed no significant differences. However, the total amount of protein varied - the two batches of

DD-*M. vaccae* contained 34% and 55% protein, whereas DD-*M. tuberculosis* and DD-*M. smegmatis* contained 79% and 72% protein, respectively.

TABLE 10
Amino Acid Composition of
Delipidated and Deglycolipidated Mycobacteria

Amino Acid	DD- <i>M.vaccae</i> Batch 1	DD- <i>M.vaccae</i> Batch 2	DD- <i>M.smegmatis</i>	DD- <i>M.tuberculosis</i>
Asp	9.5	9.5	9.3	9.1
Thr	6.0	5.9	5.0	5.3
Ser	5.3	5.3	4.2	3.3
Glu	11.1	11.2	11.1	12.5
Pro	6.1	5.9	7.5	5.2
Gly	9.9	9.7	9.4	9.8
Ala	14.6	14.7	14.6	14.2
Cys	0.5	0.5	0.3	0.5
Val	6.3	6.4	7.2	7.8
Met	1.9	1.9	1.9	1.9
Ile	3.6	3.5	4.1	4.7
Leu	7.8	7.9	8.2	8.3
Tyr	1.4	1.7	1.8	1.8
Phe	4.2	4.0	3.2	3.0
His	1.9	1.8	2.0	1.9
Lys	4.1	4.0	4.1	4.2
Arg	5.8	5.9	6.2	6.4
Total % Protein	55.1	33.8	72.1	78.5

Analysis of the monosaccharide composition shows significant differences between DD-*M. vaccae*, and DD-*M. tuberculosis* and DD-*M. smegmatis*. The monosaccharide composition of two batches of DD-*M. vaccae* was the same and differed from that of DD-*M. tuberculosis* and *M. smegmatis*. Specifically, DD-*M. vaccae* was found to contain free

glucose while both DD-*M. tuberculosis* and *M. smegmatis* contain glycerol, as shown in Table 11.

TABLE 11

Alditol Acetate	wt%	mol%
DD-M.vaccae		
Batch 1		
Inositol	0.0	0.0
Arabinose	54.7	59.1
Mannose	1.7	1.5
Glucose	31.1	28.1
Galactose	<u>12.5</u>	<u>11.3</u>
	100.0	100.0
DD-M.vaccae		
Batch 2		
Inositol	0.0	0.0
Arabinose	51.0	55.5
Mannose	2.0	1.8
Glucose	34.7	31.6
Galactose	<u>12.2</u>	<u>11.1</u>
	100.0	100.0
DD-M.smeg		
Inositol	0.0	0.0
Glycerol	15.2	15.5
Arabinose	69.3	70.7
Xylose	3.9	4.0
Mannose	2.2	1.9
Glucose	0.0	0.0
Galactose	<u>9.4</u>	<u>8.0</u>
	100.0	100.0
DD-Mtb		
Inositol	0.0	0.0
Glycerol	9.5	9.7
Arabinose	69.3	71.4
Mannose	3.5	3.0
Glucose	1.5	1.3
Galactose	<u>12.4</u>	<u>10.7</u>
	96.2	96.0

***M. vaccae* glycolipids**

The pooled 50% ethanol extracts described above were dried by rotary evaporation, redissolved in water, and freeze-dried. The amount of glycolipid recovered was 1.2% of the

starting wet weight of *M. vaccae* used. For bioassay, the glycolipids were dissolved in phosphate-buffered saline.

EXAMPLE 8

IMMUNE MODULATING PROPERTIES OF DELIPIDATED AND DEGLYCOLIPIDATED *M. VACCAE* AND RECOMBINANT PROTEINS FROM *M. VACCAE*

This example illustrates the immune modulating properties of different constituents of *M. vaccae*.

Production of Interleukin-12 from macrophages

Whole heat-killed *M. vaccae* and DD-*M. vaccae* were shown to have different cytokine stimulation properties. The stimulation of a Th1 immune response is enhanced by the production of interleukin-12 (IL-12) from macrophages. The ability of different *M. vaccae* preparations to stimulate IL-12 production was demonstrated as follows.

A group of C57BL/6J mice were injected intraperitoneally with DIFCO thioglycolate and after three days, peritoneal macrophages were collected and placed in cell culture with interferon-gamma for three hours. The culture medium was replaced and various concentrations of whole heat-killed (autoclaved) *M. vaccae*, lyophilized *M. vaccae*, DD-*M. vaccae* and *M. vaccae* glycolipids, prepared as described above, were added. After a further three days at 37 °C, the culture supernatants were assayed for the presence of IL-12 produced by macrophages. As shown in Fig. 4, the *M. vaccae* preparations stimulated the production of IL-12 from macrophages.

By contrast, these same *M. vaccae* preparations were examined for the ability to stimulate interferon-gamma production from Natural Killer (NK) cells. Spleen cells were prepared from Severe Combined Immunodeficient (SCID) mice. These populations contain 75-80% NK cells. The spleen cells were incubated at 37 °C in culture with different concentrations of heat-killed *M. vaccae*, DD-*M. vaccae*, or *M. vaccae* glycolipids. The data

shown in Fig. 5 demonstrates that, while heat-killed *M. vaccae* and *M. vaccae* glycolipids stimulate production of interferon-gamma, DD-*M. vaccae* stimulated relatively less interferon-gamma. The combined data from Figs. 4 and 5 indicate that, compared with whole heat-killed *M. vaccae*, DD-*M. vaccae* is a better stimulator of IL-12 than interferon gamma.

These findings demonstrate that removal of the lipid glycolipid constituents from *M. vaccae* results in the removal of molecular components that stimulate interferon-gamma from NK cells, thereby effectively eliminating an important cell source of a cytokine that has numerous harmful side-effects. DD-*M. vaccae* thus retains Th1 immune enhancing capacity by stimulating IL-12 production, but has lost the non-specific effects that may come through the stimulation of interferon-gamma production from NK cells.

The adjuvant effect of DD-*M. vaccae* and a number of *M. vaccae* recombinant antigens of the present invention, prepared as described below, was determined by measuring stimulation of IL-12 secretion from murine peritoneal macrophages. Figs. 6A, B, and C show data from separate experiments in which groups of C57BL/6 mice (Fig. 6A), BALB/c mice (Fig. 6B) or C3H/HeJ mice (Fig. 6C) were given DIFCO thioglycolate intraperitoneally. After three days, peritoneal macrophages were collected and placed in culture with interferon-gamma for three hours. The culture medium was replaced and various concentrations of *M. vaccae* recombinant proteins GV-3 (GV-3), GV-4P (GV-4P), GVc-7 (GV-7), GV-23, GV-27, heat killed *M. vaccae*, DD-*M. vaccae* (referred to as delipidated *M. vaccae* in Figs. 6A, B and C), *M. vaccae* glycolipids or lipopolysaccharide were added. After three days at 37 °C, the culture supernatants were assayed for the presence of IL-12 produced by macrophages. As shown in Figs. 6A, B and C, the recombinant proteins and *M. vaccae* preparations stimulated the production of IL-12 from macrophages.

In a subsequent experiment, IFN γ -primed peritoneal macrophages from BALB/c mice were stimulated with 40 ug/ml of *M. vaccae* recombinant proteins in culture for 3 days and the presence of IL-12 produced by macrophages was assayed. As shown in Fig. 7, in these experiments IFN γ -primed macrophages produced IL-12 when cultured with a control protein, ovalbumin (ova). However, the recombinant proteins GV 24B, 38BP, 38AP, 27, 5, 27B, 3, 23

and 22B stimulated more than twice the amount of IL-12 detected in control macrophage cultures.

Detection of Nonspecific Immune Amplifier from Whole *M. vaccae* and the Culture Filtrate of *M. Vaccae*

M. vaccae culture supernatant (S/N), killed *M. vaccae*, delipidated *M. vaccae* and delipidated and deglycolipidated *M. vaccae* (DD-*M. vaccae*), prepared as described above, were tested for adjuvant activity in the generation of a cytotoxic T cell immune response to ovalbumin, a structurally unrelated protein, in the mouse. This anti-ovalbumin-specific cytotoxic response was detected as follows. C57BL/6 mice (2 per group) were immunized by the intraperitoneal injection of 100 µg of ovalbumin with the following test adjuvants: autoclaved *M. vaccae*; delipidated *M. vaccae*; delipidated *M. vaccae* with glycolipids also extracted (DD-*M. vaccae*) and proteins extracted with SDS; the SDS protein extract treated with Pronase (an enzyme which degrades protein); whole *M. vaccae* culture filtrate; and heat-killed *M. tuberculosis* or heat-killed *M. bovis* BCG, *M. phlei* or *M. smegmatis* or *M. vaccae* culture filtrate. After 10 days, spleen cells were stimulated *in vitro* for a further 6 days with E.G7 cells which are EL4 cells (a C57BL/6-derived T cell lymphoma) transfected with the ovalbumin gene and thus express ovalbumin. The spleen cells were then assayed for their ability to kill non-specifically EL4 target cells or to kill specifically the E.G7 ovalbumin expressing cells. Killing activity was detected by the release of ⁵¹Chromium with which the EL4 and E.G7 cells have been labelled (100 µCi per 2x10⁶), prior to the killing assay. Killing or cytolytic activity is expressed as % specific lysis using the formula:

$$\frac{\text{cpm in test cultures} - \text{cpm in control cultures}}{\text{total cpm} - \text{cpm in control cultures}} \times 100\%$$

It is generally known that ovalbumin-specific cytotoxic cells are generated only in mice immunized with ovalbumin with an adjuvant but not in mice immunized with ovalbumin alone.

The diagrams that make up Fig. 7 show the effect of various *M. vaccae* derived adjuvant preparations on the generation of cytotoxic T cells to ovalbumin in C57BL/6 mice. As shown in Fig. 7A, cytotoxic cells were generated in mice immunized with (i) 10 µg, (ii) 100 µg or (iii) 1 mg of autoclaved *M. vaccae* or (iv) 75 µg of *M. vaccae* culture filtrate. Fig. 7B shows that cytotoxic cells were generated in mice immunized with (i) 1 mg whole autoclaved *M. vaccae* or (ii) 1 mg delipidated and deglycolipidated (DD-) *M. vaccae*. As shown in Fig. 7C(i), cytotoxic cells were generated in mice immunized with 1 mg whole autoclaved *M. vaccae*; Fig. 7C(ii) shows the active material in *M. vaccae* soluble proteins extracted with SDS from DD-*M. vaccae*. Fig. 7C(iii) shows that active material in the adjuvant preparation of Fig. 7C(ii) was destroyed by treatment with the proteolytic enzyme Pronase. By way of comparison, 100 µg of the SDS-extracted proteins had significantly stronger immune-enhancing ability (Fig. 7C(ii)) than did 1 mg whole autoclaved *M. vaccae* (Fig. 7C(i)).

Mice immunized with 1 mg heat-killed *M. vaccae* (Fig. 7D(i)) generated cytotoxic cells to ovalbumin, but mice immunized separately with 1 mg heat-killed *M. tuberculosis* (Fig. 7D(ii)), 1 mg *M. bovis* BCG (Fig. 7D(iii)), 1 mg *M. phlei* (Fig. 7D(iv)), or 1 mg *M. smegmatis* (Fig. 7D(v)) failed to generate cytotoxic cells.

These findings demonstrate that heat-killed *M. vaccae* and DD-*M. vaccae* have adjuvant properties not seen in other mycobacteria. Furthermore, delipidation and deglycolipidation of *M. vaccae* removes an NK cell-stimulating activity but does not result in a loss of T-cell stimulating activity.

In a separate experiment, mice immunised with ovalbumin plus 200 µg of DD-*M. vaccae* depleted of mycolic acids and arabinogalactan, were also able to generate cytotoxic cells (28% to 46% maximum specific lysis compared with <8% specific lysis for control mice immunised with ovalbumin alone).

The *M. vaccae* culture filtrate described above was fractionated by iso-electric focusing and the fractions assayed for adjuvant activity in the anti-ovalbumin-specific cytotoxic response assay in C57BL/6 mice as described above. Peak adjuvant activities were

demonstrated in fractions corresponding to pI of 4.2-4.32 (fraction nos. 7-9), 4.49-4.57 (fraction nos. 13-17) and 4.81-5.98 (fraction nos. 23-27).

Identification of proteins in DD-*M. vaccae* by antibodies

BALB/c mice were immunised intra-peritoneally with 50 ug of DD-*M. vaccae* once a week for 5 weeks. At the 6th week mice were sacrificed and their serum collected. The sera were tested for antibodies to recombinant *M. vaccae*-derived proteins, prepared as described below, in standard enzyme-linked immunoassays.

The antisera did not react with several *M. vaccae* recombinant proteins nor with ovalbumin, which served as an irrelevant negative control protein in the enzyme-linked assays (data not shown). Antisera from mice immunised with DD-*M. vaccae* reacted with 12 *M. vaccae*-derived GV antigens. The results are shown in Table 12 below. The antisera thus identified GV3, 5P, 5, 7, 9, 22B, 24, 27, 27A, 27B, 33 and 45 as being present in DD-*M. vaccae*.

TABLE 12
Reactivity of DD-*M. vaccae* antiserum with *M. vaccae*-derived GV antigens

GV Antigen	3	5P	5	7	9	22B	24	27	27A	27B	33	45
Reactivity*	10 ³	10 ³	10 ³	10 ²	10 ⁴	10 ³	10 ⁴	10 ⁶	10 ⁵	10 ⁶	10 ⁴	10 ⁴

*Expressed as highest dilution of serum from DD-*M. vaccae* immunised mice showing greater activity than serum from non-immunised mice.

Proteins in DD-*M. vaccae* identified by T cell responses

BALB/c mice were injected in each footpad with 100 ug DD-*M. vaccae* in combination with incomplete Freund's adjuvant and 10 days later were sacrificed to obtain popliteal lymph node cells. The cells from immunized and non-immunized control mice were stimulated *in vitro* with recombinant *M. vaccae*-derived GV proteins. After 3 days, cell proliferation and IFN γ production were assessed.

T cell proliferative responses of lymph node cells from DD-*M.vaccae* immunized mice to GV proteins.

Lymph node cells from DD-*M. vaccae*-immunized mice did not proliferate in response to an irrelevant protein, ovalbumin, (data not shown). As shown in Table 13, lymph node cells from immunized mice showed proliferative responses to GV 3, 7, 9, 23, 27, 27B, and 33. The corresponding cells from non-immunized mice did not proliferate in response to these GV proteins suggesting that mice immunized with DD-*M. vaccae* have been immunized with these proteins. Thus, the *M.vaccae* derived proteins GV 3, 7, 9, 23, 27, 27B and 33 are likely to be present in DD-*M.vaccae*.

TABLE 13

Proliferative responses of lymph node cells from DD-*M.vaccae*-immunised mice and control mice to GV proteins *in vitro*

GV protein	Stimulation index* observed in the presence of GV proteins at 50 µg/ml	
	DD- <i>M.vaccae</i> immunised mice	Control mice
GV3	4.63	1.52
GV7	3.32	1.27
GV9	6.48	2.64
GV23	4.00	1.76
GV27	5.13	1.40
GV27B	7.52	1.48
GV33	3.31	1.45

*Stimulation index = cpm from tritiated Thymidine uptake in presence of GV protein/cpm in absence of GV protein

IFN γ production by lymph node cells from DD-*M. vaccae* immunized mice following *in vitro* challenge with GV proteins

Lymph node cells from non-immunized mice did not produce IFN γ upon stimulation with GV proteins. As shown in Table 14 below, lymph node cells from DD-*M.vaccae* immunized mice secrete IFN γ in a dose dependent manner when stimulated with GV 3, 5, 23, 27A, 27B, 33, 45 or 46, suggesting that the mice have been immunized with these proteins. No IFN γ production was detectable when cells from immunized mice were stimulated with the irrelevant protein, ovalbumin (data not shown). The proteins GV 3, 5, 23, 27A, 27B, 33, 45 and 46 are thus likely to be present in DD-*M. vaccae*.

TABLE 14

Production of IFN γ by popliteal lymph node cells from DD-*M.vaccae*-immunised mice following *in vitro* challenge with GV protein

GV protein or control	IFN γ (ng/ml)		
	Dose of GV protein used <i>in vitro</i> (μ g/ml)		
	50	10	2
GV-3	8.22 \pm 3.73	ND	ND
GV-4P	ND	ND	ND
GV-5	8.90 \pm 4.54	0.57 \pm 0.40	ND
GV-5P	ND	ND	ND
GV-7	ND	ND	ND
GV-9	ND	ND	ND
GV-13	1.64 \pm 0.40	ND	ND
GV-14	ND	ND	ND
GV-14B	ND	ND	ND
GV-22B	20.15 \pm 1.96	4.34 \pm 0.02	ND
GV-23	41.38 \pm 6.69	6.97 \pm 2.78	ND
GV-24B	ND	ND	ND
GV-27	46.86 \pm 17.14	33.06 \pm 17.61	10.14 \pm 3.01
GV-27A	7.25 \pm 4.36	ND	ND
GV-27B	100.36 \pm 37.84	33.03 \pm 7.54	14.33 \pm 1.01
GV-29	5.93 \pm 0.47	ND	ND
GV-33	9.82 \pm 4.64	ND	ND
GV-38AP	1.44 \pm 1.20	ND	ND
GV-38BP	5.62 \pm 0.70	ND	ND
GV-42	ND	ND	ND
GV-44	ND	ND	ND

DD- <i>M.vaccae</i>	109.59 ± 15.48	90.23 ± 6.48	65.16 ± 3.68
<i>M. vaccae</i>	68.89 ± 4.38	67.91 ± 7.92	48.92 ± 3.86

ND = Not Detectable

Proteins in DD-*M.vaccae* as non-specific immune amplifiers

In subsequent experiments, the five proteins GV27, 27A, 27B, 23 and 45 were used as non-specific immune amplifiers with ovalbumin antigen to immunize mice as described above in Example 6. As shown in Figure 12, 50 ug of any one of the recombinant proteins GV27, 27A, 27B, 23 and 45, when injected with 50-100 ug of ovalbumin, demonstrated adjuvant properties in being able to generate cytotoxic cells to ovalbumin.

EXAMPLE 9

AUTOCLAVED *M. VACCAE* GENERATES CYTOTOXIC CD8 T CELLS AGAINST *M. TUBERCULOSIS* INFECTED MACROPHAGES

This example illustrates the ability of killed *M. vaccae* to stimulate cytotoxic CD8 T cells which preferentially kill macrophages that have been infected with *M. tuberculosis*.

Mice were immunized by the intraperitoneal injection of 500 µg of killed *M. vaccae* which was prepared as described in Example 1. Two weeks after immunization, the spleen cells of immunized mice were passed through a CD8 T cell enrichment column (R&D Systems, St. Paul, MN, USA). The spleen cells recovered from the column have been shown to be enriched up to 90% CD8 T cells. These T cells, as well as CD8 T cells from spleens of non-immunized mice, were tested for their ability to kill uninfected macrophages or macrophages which have been infected with *M. tuberculosis*.

Macrophages were obtained from the peritoneal cavity of mice five days after they have been given 1 ml of 3% thioglycolate intraperitoneally. The macrophages were infected overnight with *M. tuberculosis* at the ratio of 2 mycobacteria per macrophage. All macrophage preparations were labelled with ⁵¹Chromium at 2 µCi per 10⁴ macrophages. The macrophages were then cultured with CD8 T cells overnight (16 hours) at killer to target

ratios of 30:1. Specific killing was detected by the release of ^{51}Cr and expressed as % specific lysis, calculated as in Example 5.

The production of IFN- γ and its release into medium after 3 days of co-culture of CD8 T cells with macrophages was measured using an enzyme-linked immunosorbent assay (ELISA). ELISA plates were coated with a rat monoclonal antibody directed to mouse IFN- γ (Pharmigen, San Diego, CA, USA) in PBS for 4 hours at 4 °C. Wells were blocked with PBS containing 0.2% Tween 20 for 1 hour at room temperature. The plates were then washed four times in PBS containing 0.2% Tween 20, and samples diluted 1:2 in culture medium in the ELISA plates were incubated overnight at room temperature. The plates were again washed, and a biotinylated monoclonal rat anti-mouse IFN- γ antibody (Pharmigen), diluted to 1 $\mu\text{g}/\text{ml}$ in PBS, was added to each well. The plates were then incubated for 1 hour at room temperature, washed, and horseradish peroxidase-coupled avidin D (Sigma A-3151) was added at a 1:4,000 dilution in PBS. After a further 1 hour incubation at room temperature, the plates were washed and OPD substrate added. The reaction was stopped after 10 min with 10% (v/v) HCl. The optical density was determined at 490 nm. Fractions that resulted in both replicates giving an OD two-fold greater than the mean OD from cells cultured in medium alone were considered positive.

As shown in Table 15, CD8 T cells from spleens of mice immunized with *M. vaccae* were cytotoxic for macrophages infected with *M. tuberculosis* and did not lyse uninfected macrophages. The CD8 T cells from non-immunized mice did not lyse macrophages. CD8 T cells from naive or non-immunized mice do produce IFN- γ when cocultured with infected macrophages. The amount of IFN- γ produced in coculture was greater with CD8 T cells derived from *M. vaccae* immunized mice.

TABLE 15
EFFECT WITH *M. TUBERCULOSIS* INFECTED
AND UNINFECTED MACROPHAGES

CD8 T cells	% Specific Lysis of Macrophages		IFN- γ (ng/ml)	
	uninfected	infected	uninfected	infected
Control	0	0	0.7	24.6
<i>M. vaccae</i> Immunized	0	95	2.2	43.8

EXAMPLE 10
PURIFICATION AND CHARACTERIZATION OF POLYPEPTIDES
FROM *M. VACCAE* CULTURE FILTRATE

This example illustrates the preparation of *M. vaccae* soluble proteins from culture filtrate. Unless otherwise noted, all percentages in the following example are weight per volume.

M. vaccae (ATCC Number 15483) was cultured in sterile Medium 90 at 37 °C. The cells were harvested by centrifugation, and transferred into sterile Middlebrook 7H9 medium with glucose at 37 °C for one day. The medium was then centrifuged (leaving the bulk of the cells) and filtered through a 0.45 μ m filter into sterile bottles.

The culture filtrate was concentrated by lyophilization, and redissolved in MilliQ water. A small amount of insoluble material was removed by filtration through a 0.45 μ m membrane. The culture filtrate was desalted by membrane filtration in a 400 ml Amicon stirred cell which contained a 3kDa molecular weight cut-off (MWCO) membrane. The pressure was maintained at 50 psi using nitrogen gas. The culture filtrate was repeatedly concentrated by membrane filtration and diluted with water until the conductivity of the

sample was less than 1.0 mS. This procedure reduced the 20 l volume to approximately 50 ml. Protein concentrations were determined by the Bradford protein assay (Bio-Rad, Hercules, CA, USA).

The desalted culture filtrate was fractionated by ion exchange chromatography on a column of Q-Sepharose (Pharmacia Biotech, Uppsala, Sweden) (16 X 100 mm) equilibrated with 10mM Tris HCl buffer pH 8.0. Polypeptides were eluted with a linear gradient of NaCl from 0 to 1.0 M in the above buffer system. The column eluent was monitored at a wavelength of 280 nm.

The pool of polypeptides eluting from the ion exchange column was concentrated in a 400 ml Amicon stirred cell which contained a 3 kDa MWCO membrane. The pressure was maintained at 50 psi using nitrogen gas. The polypeptides were repeatedly concentrated by membrane filtration and diluted with 1% glycine until the conductivity of the sample was less than 0.1 mS.

The purified polypeptides were then fractionated by preparative isoelectric focusing in a Rotofor device (Bio-Rad, Hercules, CA, USA). The pH gradient was established with a mixture of Ampholytes (Pharmacia Biotech) comprising 1.6% pH 3.5-5.0 Ampholytes and 0.4% pH 5.0 - 7.0 Ampholytes. Acetic acid (0.5 M) was used as the anolyte, and 0.5 M ethanolamine as the catholyte. Isoelectric focusing was carried out at 12W constant power for 6 hours, following the manufacturer's instructions. Twenty fractions were obtained.

Fractions from isoelectric focusing were combined, and the polypeptides were purified on a Vydac C4 column (Separations Group, Hesperia, CA, USA) 300 Angstrom pore size, 5 micron particle size (10 x 250 mm). The polypeptides were eluted from the column with a linear gradient of acetonitrile (0-80% v/v) in 0.05% (v/v) trifluoroacetic acid (TFA). The flow-rate was 2.0 ml/min and the HPLC eluent was monitored at 220 nm. Fractions containing polypeptides were collected to maximize the purity of the individual samples.

Relatively abundant polypeptide fractions were rechromatographed on a Vydac C4 column (Separations Group) 300 Angstrom pore size, 5 micron particle size (4.6 x 250 mm). The polypeptides were eluted from the column with a linear gradient from 20-60% (v/v) of acetonitrile in 0.05% (v/v) TFA at a flow-rate of 1.0 ml/min. The column eluent was

monitored at 220 nm. Fractions containing the eluted polypeptides were collected to maximise the purity of the individual samples. Approximately 20 polypeptide samples were obtained and they were analysed for purity on a polyacrylamide gel according to the procedure of Laemmli (Laemmli, U. K., Nature 277:680-685, 1970).

The polypeptide fractions which were shown to contain significant contamination were further purified using a Mono Q column (Pharmacia Biotech) 10 micron particle size (5 x 50 mm) or a Vydac Diphenyl column (Separations Group) 300 Angstrom pore size, 5 micron particle size (4.6 x 250 mm). From a Mono Q column, polypeptides were eluted with a linear gradient from 0-0.5 M NaCl in 10 mM Tris HCl pH 8.0. From a Vydac Diphenyl column, polypeptides were eluted with a linear gradient of acetonitrile (20-60% v/v) in 0.1% TFA. The flow-rate was 1.0 ml/min and the column eluent was monitored at 220 nm for both columns. The polypeptide peak fractions were collected and analysed for purity on a 15% polyacrylamide gel as described above.

For sequencing, the polypeptides were individually dried onto Biobrene™ (Perkin Elmer/Applied BioSystems Division, Foster City, CA)-treated glass fiber filters. The filters with polypeptide were loaded onto a Perkin Elmer/Applied BioSystems Procise 492 protein sequencer and the polypeptides were sequenced from the amino terminal end using traditional Edman chemistry. The amino acid sequence was determined for each polypeptide by comparing the retention time of the PTH amino acid derivative to the appropriate PTH derivative standards.

Internal sequences were also determined on some antigens by digesting the antigen with the endoprotease Lys-C, or by chemically cleaving the antigen with cyanogen bromide. Peptides resulting from either of these procedures were separated by reversed-phase HPLC on a Vydac C18 column using a mobile phase of 0.05% (v/v) trifluoroacetic acid with a gradient of acetonitrile containing 0.05% (v/v) TFA (1%/min). The eluent was monitored at 214 nm. Major internal peptides were identified by their UV absorbance, and their N-terminal sequences were determined as described above.

Using the procedures described above, six soluble *M. vaccae* antigens, designated GVC-1, GVC-2, GVC-7, GVC-13, GVC-20 and GVC-22, were isolated. Determined N-terminal

and internal sequences for GVc-1 are shown in SEQ ID NOS: 1, 2 and 3, respectively; the N-terminal sequence for GVc-2 is shown in SEQ ID NO: 4; internal sequences for GVc-7 are shown in SEQ ID NOS: 5-8; internal sequences for GVc-13 are shown in SEQ ID NOS: 9-11; internal sequence for GVc-20 is shown in SEQ ID NO: 12; and N-terminal and internal sequences for GVc-22 are shown in SEQ ID NO: 56-59, respectively. Each of the internal peptide sequences provided herein begins with an amino acid residue which is assumed to exist in this position in the polypeptide, based on the known cleavage specificity of cyanogen bromide (Met) or Lys-C (Lys).

Three additional polypeptides, designated GVc-16, GVc-18 and GVc-21, were isolated employing a preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) purification step in addition to the preparative isoelectric focusing procedure described above. Specifically, fractions comprising mixtures of polypeptides from the preparative isoelectric focusing purification step previously described were purified by preparative SDS-PAGE on a 15% polyacrylamide gel. The samples were dissolved in reducing sample buffer and applied to the gel. The separated proteins were transferred to a polyvinylidene difluoride (PVDF) membrane by electroblotting in 10 mM 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) buffer pH 11 containing 10% (v/v) methanol. The transferred protein bands were identified by staining the PVDF membrane with Coomassie blue. Regions of the PVDF membrane containing the most abundant polypeptide species were cut out and directly introduced into the sample cartridge of the Perkin Elmer/Applied BioSystems Procise 492 protein sequencer. Protein sequences were determined as described above. The N-terminal sequences for GVc-16, GVc-18 and GVc-21 are provided in SEQ ID NOS: 13, 14 and 15, respectively.

Additional antigens, designated GVc-12, GVc-14, GVc-15, GVc-17 and GVc-19, were isolated employing a preparative SDS-PAGE purification step in addition to the chromatographic procedures described above. Specifically, fractions comprising a mixture of antigens from the Vydac C4 HPLC purification step previously described were fractionated by preparative SDS-PAGE on a polyacrylamide gel. The samples were dissolved in non-reducing sample buffer and applied to the gel. The separated proteins were transferred to a

PVDF membrane by electroblotting in 10 mM CAPS buffer, pH 11 containing 10% (v/v) methanol. The transferred protein bands were identified by staining the PVDF membrane with Coomassie blue. Regions of the PVDF membrane containing the most abundant polypeptide species were cut out and directly introduced into the sample cartridge of the Perkin Elmer/Applied BioSystems Procise 492 protein sequencer. Protein sequences were determined as described above. The determined N-terminal sequences for GVc-12, GVc-14, GVc-15, GVc-17 and GVc-19 are provided in SEQ ID NOS: 16-20, respectively.

All of the above amino acid sequences were compared to known amino acid sequences in the SwissProt data base (version R32) using the GeneAssist system. No significant homologies to the amino acid sequences GVc-2 to GVc-22 were obtained. The amino acid sequence for GVc-1 was found to bear some similarity to sequences previously identified from *M. bovis* and *M. tuberculosis*. In particular, GVc-1 was found to have some homology with *M. tuberculosis* MPT83, a cell surface protein, as well as MPT70. These proteins form part of a protein family (Harboe et al., *Scand. J. Immunol.* 42:46-51, 1995).

Subsequent studies led to the isolation of DNA sequences for GVc-13, GVc-14 and GVc-22 (SEQ ID NO: 142, 107 and 108, respectively). The corresponding predicted amino acid sequences for GVc-13, GVc-14 and GVc-22 are provided in SEQ ID NO: 143, 109 and 110, respectively. The determined DNA sequence for the full length gene encoding GVc-13 is provided in SEQ ID NO: 195, with the corresponding predicted amino acid sequence being provided in SEQ ID NO: 196.

Further studies with GVc-22 suggested that only a part of the gene encoding GVc-22 was cloned. When sub-cloned into the expression vector pET16, no protein expression was obtained. Subsequent screening of the *M. vaccae* *Bam*HI genomic DNA library with the incomplete gene fragment led to the isolation of the complete gene encoding GVc-22. To distinguish between the full-length clone and the partial GVc-22, the antigen expressed by the full-length gene was called GV-22B. The determined nucleotide sequence of the gene encoding GV-22B and the predicted amino acid sequence are provided in SEQ ID NOS: 144 and 145 respectively.

Amplification primers AD86 and AD112 (SEQ ID NO: 60 and 61, respectively) were designed from the amino acid sequence of GVc-1 (SEQ ID NO: 1) and the *M. tuberculosis* MPT70 gene sequence. Using these primers, a 310 bp fragment was amplified from *M. vaccae* genomic DNA and cloned into *EcoRV*-digested vector pBluescript II SK⁺ (Stratagene). The sequence of the cloned insert is provided in SEQ ID NO: 62. The insert of this clone was used to screen a *M. vaccae* genomic DNA library constructed in lambda ZAP-Express (Stratagene, La Jolla, CA). The clone isolated contained an open reading frame with homology to the *M. tuberculosis* antigen MPT83 and was re-named GV-1/83. This gene also had homology to the *M. bovis* antigen MPB83. The determined nucleotide sequence and predicted amino acid sequences are provided in SEQ ID NOS: 146 and 147 respectively.

From the amino acid sequences provided in SEQ ID NOS: 1 and 2, degenerate oligonucleotides EV59 and EV61 (SEQ ID NOS: 148 and 149 respectively) were designed. Using PCR, a 100 bp fragment was amplified, cloned into plasmid pBluescript II SK⁺ and sequenced (SEQ ID NO: 150) following standard procedures (Sambrook et al. *Ibid*). The cloned insert was used to screen a *M. vaccae* genomic DNA library constructed in lambda ZAP-Express. The clone isolated had homology to *M. tuberculosis* antigen MPT70 and *M. bovis* antigen MPB70, and was named GV-1/70. The determined nucleotide sequence and predicted amino acid sequence for GV-1/70 are provided in SEQ ID NOS: 151 and 152 respectively.

For expression and purification, the genes encoding GV1/83, GV1/70, GVc-13, GVc-14 and GV-22B were sub-cloned into the expression vector pET16 (Novagen, Madison, WI). Expression and purification were performed according to the manufacturer's protocol.

The purified polypeptides were screened for the ability to induce T-cell proliferation and IFN- γ in peripheral blood cells from immune human donors. These donors were known to be PPD (purified protein derivative from *M. tuberculosis*) skin test positive and their T cells were shown to proliferate in response to PPD. Donor PBMCs and crude soluble proteins from *M. vaccae* culture filtrate were cultured in medium comprising RPMI 1640 supplemented with 10% (v/v) autologous serum, penicillin (60 μ g/ml), streptomycin (100 μ g/ml), and glutamine (2 mM).

After 3 days, 50 μ l of medium was removed from each well for the determination of IFN- γ levels, as described below. The plates were cultured for a further 4 days and then pulsed with 1 μ Ci/well of tritiated thymidine for a further 18 hours, harvested and tritium uptake determined using a scintillation counter. Fractions that stimulated proliferation in both replicates two-fold greater than the proliferation observed in cells cultured in medium alone were considered positive.

IFN- γ was measured using an enzyme-linked immunosorbent assay (ELISA). ELISA plates were coated with a mouse monoclonal antibody directed to human IFN- γ (Endogen, Woburn, MA) 1 μ g/ml phosphate-buffered saline (PBS) for 4 hours at 4 °C. Wells were blocked with PBS containing 0.2% Tween 20 for 1 hour at room temperature. The plates were then washed four times in PBS/0.2% Tween 20, and samples diluted 1:2 in culture medium in the ELISA plates were incubated overnight at room temperature. The plates were again washed, and a biotinylated polyclonal rabbit anti-human IFN- γ serum (Endogen), diluted to 1 μ g/ml in PBS, was added to each well. The plates were then incubated for 1 hour at room temperature, washed, and horseradish peroxidase-coupled avidin A (Vector Laboratories, Burlingame, CA) was added at a 1:4,000 dilution in PBS. After a further 1 hour incubation at room temperature, the plates were washed and orthophenylenediamine (OPD) substrate added. The reaction was stopped after 10 min with 10% (v/v) HCl. The optical density (OD) was determined at 490 nm. Fractions that resulted in both replicates giving an OD two-fold greater than the mean OD from cells cultured in medium alone were considered positive.

Examples of polypeptides containing sequences that stimulate peripheral blood mononuclear cells (PBMC) T cells to proliferate and produce IFN- γ are shown in Table 16, wherein (-) indicates a lack of activity, (+/-) indicates polypeptides having a result less than twice higher than background activity of control media, (+) indicates polypeptides having activity two to four times above background, and (++) indicates polypeptides having activity greater than four times above background.

TABLE 16

Antigen	Proliferation	IFN- γ
GVC-1	++	+/-
GVC-2	+	++
GVC-7	+/-	-
GVC-13	+	++
GVC-14	++	+
GVC-15	+	+
GVC-20	+	+

EXAMPLE 11

PURIFICATION AND CHARACTERISATION OF POLYPEPTIDES
FROM *M. VACCAE* CULTURE FILTRATE BY
2-DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORESIS

M. vaccae soluble proteins were isolated from culture filtrate using 2-dimensional polyacrylamide gel electrophoresis as described below. Unless otherwise noted, all percentages in the following example are weight per volume.

M. vaccae (ATCC Number 15483) was cultured in sterile Medium 90 at 37 °C. *M. tuberculosis* strain H37Rv (ATCC number 27294) was cultured in sterile Middlebrook 7H9 medium with Tween 80 and oleic acid/albumin/dextrose/catalase additive (Difco Laboratories, Detroit, Michigan). The cells were harvested by centrifugation, and transferred into sterile Middlebrook 7H9 medium with glucose at 37 °C for one day. The medium was then centrifuged (leaving the bulk of the cells) and filtered through a 0.45 μ m filter into sterile bottles. The culture filtrate was concentrated by lyophilisation, and redissolved in MilliQ water. A small amount of insoluble material was removed by filtration through a 0.45 μ m membrane filter.

The culture filtrate was desalted by membrane filtration in a 400 ml Amicon stirred cell which contained a 3 kDa MWCO membrane. The pressure was maintained at 60 psi using nitrogen gas. The culture filtrate was repeatedly concentrated by membrane filtration and diluted with water until the conductivity of the sample was less than 1.0 mS. This procedure reduced the 20 l volume to approximately 50 ml. Protein concentrations were determined by the Bradford protein assay (Bio-Rad, Hercules, CA, USA).

The desalted culture filtrate was fractionated by ion exchange chromatography on a column of Q-Sepharose (Pharmacia Biotech) (16 x 100 mm) equilibrated with 10mM TrisHCl buffer pH 8.0. Polypeptides were eluted with a linear gradient of NaCl from 0 to 1.0 M in the above buffer system. The column eluent was monitored at a wavelength of 280 nm.

The pool of polypeptides eluting from the ion exchange column were fractionated by preparative 2D gel electrophoresis. Samples containing 200-500 µg of polypeptide were made 8M in urea and applied to polyacrylamide isoelectric focusing rod gels (diameter 2mm, length 150 mm, pH 5-7). After the isoelectric focusing step, the first dimension gels were equilibrated with reducing buffer and applied to second dimension gels (16% polyacrylamide). Polypeptides from the second dimension separation were transferred to PVDF membranes by electroblotting in 10mM CAPS buffer pH 11 containing 10% (v/v) methanol. The PVDF membranes were stained for protein with Coomassie blue. Regions of PVDF containing polypeptides of interest were cut out and directly introduced into the sample cartridge of the Perkin Elmer/Applied BioSystems Procise 492 protein sequencer. The polypeptides were sequenced from the amino terminal end using traditional Edman chemistry. The amino acid sequence was determined for each polypeptide by comparing the retention time of the PTH amino acid derivative to the appropriate PTH derivative standards. Using these procedures, eleven polypeptides, designated GVs-1, GVs-3, GVs-4, GVs-5, GVs-6, GVs-8, GVs-9, GVs-10, GVs-11, GV-34 and GV-35 were isolated. The determined N-terminal sequences for these polypeptides are shown in SEQ ID NOS: 21-29, 63 and 64, respectively. Using the purification procedure described above, more protein was purified to extend the amino acid sequence previously obtained for GVs-9. The extended amino acid sequence for GVs-9 is provided in SEQ ID NO: 65. Further studies resulted in the isolation

of DNA sequences for GVs-9 (SEQ ID NO: 111) and GV-35 (SEQ ID NO: 155). The corresponding predicted amino acid sequences are provided in SEQ ID NO: 112 and 156, respectively. An extended DNA sequence for GVs-9 is provided in SEQ ID NO: 153, with the corresponding predicted amino acid sequence being provided in SEQ ID NO: 154. The predicted amino acid sequence for GVs-9 has been amended in SEQ ID NO: 197.

All of these amino acid sequences were compared to known amino acid sequences in the SwissProt data base (version R35 plus update). No significant homologies were obtained, with the exceptions of GVs-3, GVs-4, GVs-5 and GVs-9. GVs-9 was found to bear some homology to two previously identified *M. tuberculosis* proteins, namely *M. tuberculosis* cutinase precursor and an *M. tuberculosis* hypothetical 22.6 kDa protein. GVs-3, GVs-4 and GVs-5 were found to bear some similarity to the antigen 85A and 85B proteins from *M. leprae* (SEQ ID NOS: 30 and 31, respectively), *M. tuberculosis* (SEQ ID NOS: 32 and 33, respectively) and *M. bovis* (SEQ ID NOS: 34 and 35, respectively), and the antigen 85C proteins from *M. leprae* (SEQ ID NO: 36) and *M. tuberculosis* (SEQ ID NO: 37).

EXAMPLE 12

DNA CLONING STRATEGY FOR THE *M. VACCAE*

ANTIGEN 85 SERIES

Probes for antigens 85A, 85B, and 85C were prepared by polymerase chain reaction (PCR) using degenerate oligonucleotides (SEQ ID NOS: 38 and 39) designed to regions of antigen 85 genomic sequence that are conserved between family members in a given mycobacterial species, and between mycobacterial species. These oligonucleotides were used under reduced stringency conditions to amplify target sequences from *M. vaccae* genomic DNA. An appropriately-sized 485 bp band was identified, purified, and cloned into T-tailed pBluescript II SK (Stratagene, La Jolla, CA). Twenty-four individual colonies were screened at random for the presence of the antigen 85 PCR product, then sequenced using the Perkin Elmer/Applied Biosystems Model 377 automated sequencer and the M13-based primers, T3 and T7. Homology searches of the GenBank databases showed that twenty-three clones contained insert with significant homology to published antigen 85 genes from *M.*

tuberculosis and *M. bovis*. Approximately half were most homologous to antigen 85C gene sequences, with the remainder being more similar to antigen 85B sequences. In addition, these two putative *M. vaccae* antigen 85 genomic sequences were 80% homologous to one another. Because of this high similarity, the antigen 85C PCR fragment was chosen to screen *M. vaccae* genomic libraries at low stringency for all three antigen 85 genes.

An *M. vaccae* genomic library was created in lambda Zap-Express (Stratagene, La Jolla, CA) by cloning *Bam*HI partially-digested *M. vaccae* genomic DNA into similarly-digested λ vector, with 3.4×10^5 independent plaque-forming units resulting. For screening purposes, twenty-seven thousand plaques from this non-amplified library were plated at low density onto eight 100 cm² plates. For each plate, duplicate plaque lifts were taken onto Hybond-N⁺ nylon membrane (Amersham International, United Kingdom), and hybridised under reduced-stringency conditions (55 °C) to the radiolabelled antigen 85C PCR product. Autoradiography demonstrated that seventy-nine plaques consistently hybridised to the antigen 85C probe under these conditions. Thirteen positively-hybridising plaques were selected at random for further analysis and removed from the library plates, with each positive clone being used to generate secondary screening plates containing about two hundred plaques. Duplicate lifts of each plate were taken using Hybond-N⁺ nylon membrane, and hybridised under the conditions used in primary screening. Multiple positively-hybridising plaques were identified on each of the thirteen plates screened. Two well-isolated positive phage from each secondary plate were picked for further analysis. Using *in vitro* excision, twenty-six plaques were converted into phagemid, and restriction-mapped. It was possible to group clones into four classes on the basis of this mapping. Sequence data from the 5' and 3' ends of inserts from several representatives of each group was obtained using the Perkin Elmer/Applied Biosystems Model 377 automated sequencer and the T3 and T7 primers. Sequence homologies were determined using BLASTN analysis of the EMBL database. Two of these sets of clones were found to be homologous to *M. bovis* and *M. tuberculosis* antigen 85A genes, each containing either the 5' or 3' ends of the *M. vaccae* gene (this gene was cleaved during library construction as it contains an internal *Bam*HI site). The remaining clones were found to contain sequences homologous to antigens 85B and 85C from a number

of mycobacterial species. To determine the remaining nucleotide sequence for each gene, appropriate subclones were constructed and sequenced. Overlapping sequences were aligned using the DNA Strider software. The determined DNA sequences for *M. vaccae* antigens 85A, 85B and 85C are shown in SEQ ID NOS: 40-42, respectively, with the predicted amino acid sequences being shown in SEQ ID NOS: 43-45, respectively.

The *M. vaccae* antigens GVs-3 and GVs-5 were expressed and purified as follows. Amplification primers were designed from the insert sequences of GVs-3 and GVs-5 (SEQ ID NO: 40 and 42, respectively) using sequence data downstream from the putative leader sequence and the 3' end of the clone. The sequences of the primers for GVs-3 are provided in SEQ ID NO: 66 and 67, and the sequences of the primers for GVs-5 are provided in SEQ ID NO: 68 and 69. A *Xho*I restriction site was added to the primers for GVs-3, and *Eco*RI and *Bam*HI restriction sites were added to the primers for GVs-5 for cloning convenience. Following amplification from genomic *M. vaccae* DNA, fragments were cloned into the appropriate site of pProEX HT prokaryotic expression vector (Gibco BRL, Life Technologies, Gaithersburg, MD) and submitted for sequencing to confirm the correct reading frame and orientation. Expression and purification of the recombinant protein was performed according to the manufacturer's protocol.

Expression of a fragment of the *M. vaccae* antigen GVs-4 (antigen 85B homolog) was performed as follows. The primers AD58 and AD59, described above, were used to amplify a 485 bp fragment from *M. vaccae* genomic DNA. This fragment was gel-purified using standard techniques and cloned into *Eco*RV-digested pBluescript containing added dTTP residues. The base sequences of inserts from five clones were determined and found to be identical to each other. These inserts had highest homology to Ag85B from *M. tuberculosis*. The insert from one of the clones was subcloned into the *Eco*RI/*Xho*I sites of pProEX HT prokaryotic expression vector (Gibco BRL), expressed and purified according to the manufacturer's protocol. This clone was renamed GV-4P because only a part of the gene was expressed. The amino acid and DNA sequences for the partial clone GV-4P are provided in SEQ ID NO: 70 and 106, respectively.

Similar to the cloning of GV-4P, the amplification primers AD58 and AD59 were used to amplify a 485 bp fragment from a clone containing GVs-5 (SEQ ID NO:42). This fragment was cloned into the expression vector pET16 and was called GV-5P. The determined nucleotide sequence and predicted amino acid sequence of GV-5P are provided in SEQ ID NOS: 157 and 158, respectively.

In subsequent studies, using procedures similar to those described above, GVs-3, GV-4P and GVs-5 were re-cloned into the alternative vector pET16 (Novagen, Madison, WI).

The ability of purified recombinant GVs-3, GV-4P and GVs-5 to stimulate proliferation of T cells and interferon- γ production in human PBL from PPD-positive, healthy donors, was assayed as described above. The results of this assay are shown in Table 17, wherein (-) indicates a lack of activity, (+/-) indicates polypeptides having a result less than twice higher than background activity of control media, (+) indicates polypeptides having activity two to four times above background, (++) indicates polypeptides having activity greater than four times above background, and ND indicates not determined.

Table 17

	Donor G97005		Donor G97006		Donor G97007		Donor G97008		Donor G97009		Donor G97010	
	Prolif	IFN γ	Prolif	IFN γ	Prolif	IFN γ	Prolif	IFN γ	Prolif	IFN γ	Prolif	IFN γ
GVs-3	++	+	ND	ND	++	++	++	++	++	+/-	+	++
GV -4P	+	+/-	ND	ND	+	++	++	++	+/-	+/-	+/-	++
GVs-5	++	++	++	++	++	++	+	++	++	+	+	++

EXAMPLE 13

DNA CLONING STRATEGY FOR *M. VACCAE* ANTIGENS

An 84 bp probe for the *M. vaccae* antigen GVc-7 was amplified using degenerate oligonucleotides designed to the determined amino acid sequence of GVc-7 (SEQ ID NOS: 5-8). This probe was used to screen a *M. vaccae* genomic DNA library as described in Example

12. The determined nucleotide sequence for GVc-7 is shown in SEQ ID NO: 46 and predicted amino acid sequence in SEQ ID NO: 47. Comparison of these sequences with those in the databank revealed homology to a hypothetical 15.8 kDa membrane protein of *M. tuberculosis*.

The sequence of SEQ ID NO: 46 was used to design amplification primers (provided in SEQ ID NO: 71 and 72) for expression cloning of the GVc-7 gene using sequence data downstream from the putative leader sequence. A *Xho*I restriction site was added to the primers for cloning convenience. Following amplification from genomic *M. vaccae* DNA, fragments were cloned into the *Xho*I-site of pProEX HT prokaryotic expression vector (Gibco BRL) and submitted for sequencing to confirm the correct reading frame and orientation. Expression and purification of the fusion protein was performed according to the manufacturer's protocol. In subsequent studies, GVc-7 was re-cloned into the vector pET16 (Novagen).

The ability of purified recombinant GVc-7 to stimulate proliferation of T-cells and stimulation of interferon- γ production in human PBL, from PPD-positive, healthy donors, was assayed as described above. The results are shown in Table 18, wherein (-) indicates a lack of activity, (+/-) indicates polypeptides having a result less than twice higher than background activity of control media, (+) indicates polypeptides having activity two to four times above background, and (++) indicates polypeptides having activity greater than four times above background.

TABLE 18

Donor	Proliferation	Interferon- γ
G97005	++	+/-
G97008	++	+
G97009	+	+/-
G97010	+/-	++

A redundant oligonucleotide probe (SEQ ID NO 73; referred to as MPG15) was designed to the GVc-8 peptide sequence shown in SEQ ID NO: 26 and used to screen a *M. vaccae* genomic DNA library using standard protocols. Two genomic clones containing genes encoding four different antigens was isolated. The determined DNA sequences for

GVs-8A (re-named GV-30), GV-8B (re-named GV-31), GV-8C (re-named GV-32) and GV-8D (re-named GV-33) are shown in SEQ ID NOS: 48-51, respectively, with the corresponding amino acid sequences being shown in SEQ ID NOS: 52-55, respectively. GV-30 contains regions showing some similarity to known prokaryotic valyl-tRNA synthetases; GV-31 shows some similarity to *M. smegmatis* aspartate semialdehyde dehydrogenase; and GV-32 shows some similarity to the *H. influenza* folylpolyglutamate synthase gene. GV-33 contains an open reading frame which shows some similarity to sequences previously identified in *M. tuberculosis* and *M. leprae*, but whose function has not been identified.

The determined partial DNA sequence for GV-33 is provided in SEQ ID NO: 74 with the corresponding predicted amino acid sequence being provided in SEQ ID NO: 75. Sequence data from the 3' end of the clone showed homology to a previously identified 40.6 kDa outer membrane protein of *M. tuberculosis*. Subsequent studies led to the isolation of a full-length DNA sequence for GV-33 (SEQ ID NO: 193). The corresponding predicted amino acid sequence is provided in SEQ ID NO: 194.

The gene encoding GV-33 was amplified from *M. vaccae* genomic DNA with primers based on the determined nucleotide sequence. This DNA fragment was cloned into *EcoR*v-digested pBluescript II SK⁺ (Stratagene), and then transferred to pET16 expression vector. Recombinant protein was purified following the manufacturer's protocol.

The ability of purified recombinant GV-33 to stimulate proliferation of T-cells and stimulation of interferon- γ production in human PBL was assayed as described above. The results are shown in Table 19, wherein (-) indicates a lack of activity, (+/-) indicates polypeptides having a result less than twice higher than background activity of control media, (+) indicates polypeptides having activity two to four times above background, and (++) indicates polypeptides having activity greater than four times above background.

TABLE 19
Stimulatory Activity of Polypeptides

Donor	Proliferation	Interferon- γ
G97005	++	+
G97006	++	++
G97007	-	+/-
G97008	+/-	-
G97009	+/-	-
G97010	+/-	++

EXAMPLE 14
ISOLATION OF PROTEINS FROM DD-*M. VACCAE*

M. vaccae bacteria were cultured, pelleted and autoclaved as described in Example 1. Culture filtrates of live *M. vaccae* refer to the supernatant from 24 hour cultures of *M. vaccae* in 7H9 medium with glucose. A delipidated form of *M. vaccae* was prepared by sonicating autoclaved *M. vaccae* for four bursts of 30 seconds on ice using the Virsonic sonicator (Virtis, Disa, USA). The material was then centrifuged (9000 rpm, 20 minutes, JA10 rotor, brake = 5). The resulting pellet was suspended in 100 ml of chloroform/methanol (2:1), incubated at room temperature for 1 hour, re-centrifuged, and the chloroform/methanol extraction repeated. The pellet was obtained by centrifugation, dried *in vacuo*, weighed and resuspended in PBS at 50 mg (dry weight) per ml as delipidated *M. vaccae*.

Glycolipids were removed from the delipidated *M. vaccae* preparation by refluxing in 50% v/v ethanol for 2 hours. The insoluble material was collected by centrifugation (10,000 rpm, JA20 rotor, 15 mins, brake = 5). The extraction with 50% v/v ethanol under reflux was repeated twice more. The insoluble material was collected by centrifugation and washed in PBS. Proteins were extracted by resuspending the pellet in 2% SDS in PBS at 56 °C for 2 hours. The insoluble material was collected by centrifugation and the extraction with 2% SDS/PBS at 56 °C was repeated twice more. The pooled SDS extracts were cooled to 4 °C, and precipitated SDS was removed by centrifugation (10,000 rpm, JA20 rotor, 15 mins, brake

= 5). Proteins were precipitated from the supernatant by adding an equal volume of acetone and incubating at -20 °C for 2 hours. The precipitated proteins were collected by centrifugation, washed in 50% v/v acetone, dried *in vacuo*, and redissolved in PBS.

The SDS-extracted proteins derived from DD-*M. vaccae* were analysed by polyacrylamide gel electrophoresis. Three major bands were observed after staining with silver. In subsequent experiments, larger amounts of SDS-extracted proteins from DD-*M. vaccae*, were analysed by polyacrylamide gel electrophoresis. The proteins, on staining with Coomassie blue, showed several bands. A protein represented by a band of approximate molecular weight of 30 kDa was designated GV-45. The determined N-terminal sequence for GV-45 is provided in SEQ ID NO: 187. A protein of approximate molecular weight of 14 kDa was designated GV-46. The determined N-terminal amino acid sequence of GV-46 is provided in SEQ ID NO: 208.

In subsequent studies, more of the SDS-extracted proteins described above were prepared by preparative SDS-PAGE on a BioRad Prep Cell (Hercules, CA). Fractions corresponding to molecular weight ranges were precipitated by trichloroacetic acid to remove SDS before assaying for adjuvant activity in the anti-ovalbumin-specific cytotoxic response assay in C57BL/6 mice as described above. The adjuvant activity was highest in the 60-70 kDa fraction. The most abundant protein in this size range was purified by SDS-PAGE blotted on to a polyvinylidene difluoride (PVDF) membrane and then sequenced. The sequence of the first ten amino acid residues is provided in SEQ ID NO:76. Comparison of this sequence with those in the gene bank as described above, revealed homology to the heat shock protein 65 (GroEL) gene from *M. tuberculosis*, indicating that this protein is an *M. vaccae* member of the GroEL family.

An expression library of *M. vaccae* genomic DNA in *Bam*H1-lambda ZAP-Express (Stratagene) was screened using sera from cynomolgous monkeys immunised with *M. vaccae* secreted proteins prepared as described above. Positive plaques were identified using a colorimetric system. These plaques were re-screened until plaques were pure following standard procedures. pBK-CMV phagemid 2-1 containing an insert was excised from the lambda ZAP Express (Stratagene) vector in the presence of ExAssist helper phage following

the manufacturer's protocol. The base sequence of the 5' end of the insert of this clone, hereinafter referred to as GV-27, was determined using Sanger sequencing with fluorescent primers on Perkin Elmer/Applied Biosystems Division automatic sequencer. The determined nucleotide sequence of the partial *M. vaccae* GroEL-homologue clone GV-27 is provided in SEQ ID NO: 77 and the predicted amino acid sequence in SEQ ID NO: 78. This clone was found to have homology to *M. tuberculosis* GroEL. A partial sequence of the 65 kDa heat shock protein of *M. vaccae* has been published by Kapur et al. (*Arch. Pathol. Lab. Med.* 119 :131-138, 1995). The nucleotide sequence of the Kapur et al. fragment is shown in SEQ ID NO: 79 and the predicted amino acid sequence in SEQ ID NO: 80.

In subsequent studies, an extended (full-length except for the predicted 51 terminal nucleotides) DNA sequence for GV-27 was obtained (SEQ ID NO: 113). The corresponding predicted amino acid sequence is provided in SEQ ID NO: 114. Further studies led to the isolation of a full-length DNA sequence for GV-27 (SEQ ID NO: 159). The corresponding predicted amino acid sequence is provided in SEQ ID NO: 160. GV-27 was found to be 93.7% identical to the *M. tuberculosis* GroEL at the amino acid level.

Two peptide fragments, comprising the N-terminal sequence (hereinafter referred to as GV-27A) and the carboxy terminal sequence of GV-27 (hereinafter referred to as GV-27B) were prepared using techniques well known in the art. The nucleotide sequences for GV-27A and GV-27B are provided in SEQ ID NO: 115 and 116, respectively, with the corresponding amino acid sequences being provided in SEQ ID NO: 117 and 118. Subsequent studies led to the isolation of an extended DNA sequence for GV-27B. This sequence is provided in SEQ ID NO: 161, with the corresponding amino acid sequence being provided in SEQ ID NO: 162. The sequence of GV-27A is 95.8% identical to the *M. tuberculosis* GroEL sequence and contains the shorter *M. vaccae* sequence of Kapur et al. discussed above. The sequence for GV-27B shows about 92.2% identity to the corresponding region of *M. tuberculosis* HSP65. Following the same protocol as for the isolation of GV-27, pBK-CMV phagemid 3-1 was isolated. The antigen encoded by this DNA was named GV-29. The determined nucleotide sequences of the 5' and 3' ends of the gene are provided in SEQ ID NOS: 163 and 164, respectively, with the predicted corresponding amino acid sequences being provided in SEQ

ID NOS: 165 and 166 respectively. GV-29 showed homology to yeast urea amidolyase. The determined DNA sequence for the full-length gene encoding GV-29 is provided in SEQ ID NO: 198, with the corresponding predicted amino acid sequence in SEQ ID NO: 199. The DNA encoding GV-29 was sub-cloned into the vector pET16 (Novagen, Madison, WI) for expression and purification according to standard protocols.

EXAMPLE 15

DNA CLONING STRATEGY FOR THE *M. VACCAE* ANTIGENS

GV-23, GV-24, GV-25, GV-26, GV-38A AND GV-38B

M. vaccae (ATCC Number 15483) was grown in sterile Medium 90 at 37 °C for 4 days and harvested by centrifugation. Cells were resuspended in 1 ml Trizol (Gibco BRL, Life Technologies, Gaithersburg, Maryland) and RNA extracted according to the standard manufacturer's protocol. *M. tuberculosis* strain H37Rv (ATCC Number 27294) was grown in sterile Middlebrook 7H9 medium with Tween 80™ and oleic acid/ albumin/dextrose/catalase additive (Difco Laboratories, Detroit, Michigan) at 37 °C and harvested under appropriate laboratory safety conditions. Cells were resuspended in 1 ml Trizol (Gibco BRL) and RNA extracted according to the manufacturer's standard protocol.

Total *M. tuberculosis* and *M. vaccae* RNA was depleted of 16S and 23S ribosomal RNA (rRNA) by hybridisation of the total RNA fraction to oligonucleotides AD10 and AD11 (SEQ ID NO: 81 and 82) complementary to *M. tuberculosis* rRNA. These oligonucleotides were designed from mycobacterial 16S rRNA sequences published by Bottger (*FEMS Microbiol. Lett.* 65:171-176, 1989) and from sequences deposited in the databanks. Depletion was done by hybridisation of total RNA to oligonucleotides AD10 and AD11 immobilised on nylon membranes (Hybond N, Amersham International, United Kingdom). Hybridisation was repeated until rRNA bands were not visible on ethidium bromide-stained agarose gels. An oligonucleotide, AD12 (SEQ ID NO: 83), consisting of 20 dATP-residues, was ligated to the 3' ends of the enriched mRNA fraction using RNA ligase. First strand cDNA synthesis was performed following standard protocols, using oligonucleotide AD7 (SEQ ID NO:84) containing a poly(dT) sequence.

The *M. tuberculosis* and *M. vaccae* cDNA was used as template for single-sided-specific PCR (3S-PCR). For this protocol, a degenerate oligonucleotide AD1 (SEQ ID NO:85) was designed based on conserved leader sequences and membrane protein sequences. After 30 cycles of amplification using primer AD1 as 5'-primer and AD7 as 3'-primer, products were separated on a urea/polyacrylamide gel. DNA bands unique to *M. vaccae* were excised and re-amplified using primers AD1 and AD7. After gel purification, bands were cloned into pGEM-T (Promega) and the base sequence determined.

Searches with the determined nucleotide and predicted amino acid sequences of band 12B21 (SEQ ID NOS: 86 and 87, respectively) showed homology to the *pota* gene of *E.coli* encoding the ATP-binding protein of the spermidine/putrescine ABC transporter complex published by Furuchi et al. (*Jnl. Biol. Chem.* 266: 20928-20933, 1991). The spermidine/putrescine transporter complex of *E.coli* consists of four genes and is a member of the ABC transporter family. The ABC (ATP-binding Cassette) transporters typically consist of four genes: an ATP-binding gene, a periplasmic, or substrate binding, gene and two transmembrane genes. The transmembrane genes encode proteins each characteristically having six membrane-spanning regions. Homologues (by similarity) of this ABC transporter have been identified in the genomes of *Haemophilus influenza* (Fleischmann et al. *Science* 269 :496-512, 1995) and *Mycoplasma genitalium* (Fraser, et al. *Science*, 270:397-403, 1995).

An *M. vaccae* genomic DNA library constructed in BamH1-digested lambda ZAP Express (Stratagene) was probed with the radiolabelled 238 bp band 12B21 following standard protocols. A plaque was purified to purity by repetitive screening and a phagemid containing a 4.5 kb insert was identified by Southern blotting and hybridisation. The nucleotide sequence of the full-length *M. vaccae* homologue of *pota* (ATP-binding protein) was identified by subcloning of the 4.5 kb fragment and base sequencing. The gene consisted of 1449 bp including an untranslated 5' region of 320 bp containing putative -10 and -35 promoter elements. The nucleotide and predicted amino acid sequences of the *M. vaccae pota* homologue are provided in SEQ ID NO: 88 and 89, respectively.

The nucleotide sequence of the *M. vaccae pota* gene was used to design primers EV24 and EV25 (SEQ ID NO: 90 and 91) for expression cloning. The amplified DNA fragment

was cloned into pProEX HT prokaryotic expression system (Gibco BRL) and expression in an appropriate *E. coli* host was induced by addition of 0.6 mM isopropylthio- β -galactoside (IPTG). The recombinant protein was named GV-23 and purified from inclusion bodies according to the manufacturer's protocol. In subsequent studies, GV-23 (SEQ ID NO: 88) was re-cloned into the alternative vector pET16 (Novagen). The amino acid sequence of SEQ ID NO: 89 contains an ATP binding site at residues 34 to 41. At residues 116 to 163 of SEQ ID NO: 89, there is a conserved region that is found in the ATP-transporter family of proteins. These findings suggest that GV-23 is an ATP binding protein.

A 322 bp *SalI*-*Bam*HI subclone at the 3'-end of the 4.5 kb insert described above showed homology to the *potd* gene, (periplasmic protein), of the spermidine/putrescine ABC transporter complex of *E. coli*. The nucleotide sequence of this subclone is shown in SEQ ID NO:92. To identify the gene, the radiolabelled insert of this subclone was used to probe a *M. vaccae* genomic DNA library constructed in the *SalI*-site of lambda Zap Express (Stratagene) following standard protocols. A clone was identified of which 1342 bp showed homology with the *potd* gene of *E. coli*. The *potd* homologue of *M. vaccae* was identified by sub-cloning and base sequencing. The determined nucleotide and predicted amino acid sequences are shown in SEQ ID NO: 93 and 94.

For expression cloning, primers EV-26 and EV-27 (SEQ ID NOS: 95-96) were designed from the determined *M. vaccae potd* homologue. The amplified fragment was cloned into pProEX HT Prokaryotic expression system (Gibco BRL). Expression in an appropriate *E. coli* host was induced by addition of 0.6 mM IPTG and the recombinant protein named GV-24. The recombinant antigen was purified from inclusion bodies according to the protocol of the supplier. In subsequent studies, GV-24 (SEQ ID NO: 93) was re-cloned into the alternative vector pET16 (Novagen).

To improve the solubility of the purified recombinant antigen, the gene encoding GV-24, but excluding the signal peptide, was re-cloned into the expression vector, employing amplification primers EV101 and EV102 (SEQ ID NOS: 167 and 168). The construct was designated GV-24B. The nucleotide sequence of GV-24B is provided in SEQ ID NO: 169

and the predicted amino acid sequence in SEQ ID NO: 170. This fragment was cloned into pET16 for expression and purification of GV-24B according to the manufacturer's protocols.

The ability of purified recombinant protein GV-23 and GV-24 to stimulate proliferation of T cells and interferon- γ production in human PBL was determined as described above. The results of these assays are provided in Table 20, wherein (-) indicates a lack of activity, (+/-) indicates polypeptides having a result less than twice higher than background activity of control media, (+) indicates polypeptides having activity two to four times above background, (++) indicates polypeptides having activity greater than four times above background, and (ND) indicates not determined.

TABLE 20

	Donor G97005		Donor G97006		Donor G97007		Donor G97008		Donor G97009		Donor G97010	
	Prolif	IFN- γ	Prolif	IFN- γ	Prolif	IFN- γ	Prolif	IFN- γ	Prolif	IFN- γ	Prolif	IFN- γ
GV-23	++	++	++	++	+	+	++	++	+	-	+	++
GV-24	++	+	++	+	ND	ND	+	+/-	+	+/-	+/-	++

Base sequence adjacent to the *M. vaccae potd* gene-homologue was found to show homology to the *potb* gene of the spermidine/putrescine ABC transporter complex of *E. coli*, which is one of two transmembrane proteins in the ABC transporter complex. The *M. vaccae potb* homologue (referred to as GV-25) was identified through further subcloning and base sequencing. The determined nucleotide and predicted amino acid sequences for GV-25 are shown in SEQ ID NOS: 97 and 98, respectively.

Further subcloning and base sequence analysis of the adjacent 509 bp failed to reveal significant homology to PotC, the second transmembrane protein of *E. coli*, and suggests that a second transmembrane protein is absent in the *M. vaccae* homologue of the ABC transporter. An open reading frame with homology to *M. tuberculosis* acetyl-CoA acetyl transferase, however, was identified starting 530 bp downstream of the transmembrane protein and the translated protein was named GV-26. The determined partial nucleotide sequence and predicted amino acid sequence for GV-26 are shown in SEQ ID NO: 99 and 100, respectively.

Using a protocol similar to that described above for the isolation of GV-23, the 3S-PCR band 12B28 (SEQ ID NO: 119) was used to screen the *M. vaccae* genomic library constructed in the BamHI-site of lambda ZAP Express (Stratagene). The clone isolated from the library contained a novel open reading frame and the antigen encoded by this gene was named GV-38A. The determined nucleotide sequence and predicted amino acid sequence of GV-38A are shown in SEQ ID NO: 120 and 121, respectively. Subsequent studies led to the isolation of an extended DNA sequence for GV-38A, provided in SEQ ID NO: 171. The corresponding amino acid sequence is provided in SEQ ID NO: 172. Comparison of these sequences with those in the gene bank, revealed some homology to an unknown *M. tuberculosis* protein previously identified in cosmid MTCY428.12. (SPTREMBL:P71915).

Upstream of the GV-38A gene, a second novel open reading frame was identified and the antigen encoded by this gene was named GV-38B. The determined 5' and 3' nucleotide sequences for GV-38B are provided in SEQ ID NO: 122 and 123, respectively, with the corresponding predicted amino acid sequences being provided in SEQ ID NO: 124 and 125, respectively. Further studies led to the isolation of the full-length DNA sequence for GV-38B, provided in SEQ ID NO: 173. The corresponding amino acid sequence is provided in SEQ ID NO: 174. This protein was found to show homology to an unknown *M. tuberculosis* protein identified in cosmid MTCY428.11 (SPTREMBL: P71914).

Both the GV-38A and GV-38B antigens were amplified for expression cloning into pET16 (Novagen). GV-38A was amplified with primers KR11 and KR12 (SEQ ID NO: 126 and 127) and GV-38B with primers KR13 and KR14 (SEQ ID NO: 128 and 129). Protein expression in the host cells BL21(DE3) was induced with 1 mM IPTG, however no protein expression was obtained from these constructs. Hydrophobic regions were identified in the N-termini of antigens GV-38A and GV-38B which may inhibit expression of these constructs. The hydrophobic region present in GV-38A was identified as a possible transmembrane motif with six membrane spanning regions. To express the antigens without the hydrophobic regions, primers KR20 for GV-38A, (SEQ ID NO: 130) and KR21 for GV-38B (SEQ ID NO: 131) were designed. The truncated GV-38A gene was amplified with primers KR20 and KR12, and the truncated GV-38B gene with KR21 and KR14. The determined nucleotide

sequences of truncated GV38A and GV-38B are shown in SEQ ID NO: 132 and 133, respectively, with the corresponding predicted amino acid sequences being shown in SEQ ID NO: 134 and 135, respectively. Extended DNA sequences for truncated GV-38A and GV-38B are provided in SEQ ID NO: 175 and 176, respectively, with the corresponding amino acid sequences being provided in SEQ ID NO: 177 and 178, respectively.

EXAMPLE 16

PURIFICATION AND CHARACTERISATION OF POLYPEPTIDES FROM *M. VACCAE* CULTURE FILTRATE BY PREPARATIVE ISOELECTRIC FOCUSING AND PREPARATIVE POLYACRYLAMIDE GEL ELECTROPHORESIS

M. vaccae soluble proteins were isolated from culture filtrate using preparative isoelectric focusing and preparative polyacrylamide gel electrophoresis as described below. Unless otherwise noted, all percentages in the following example are weight per volume.

M. vaccae (ATCC Number 15483) was cultured in 250 l sterile Medium 90 which had been fractionated by ultrafiltration to remove all proteins of greater than 10 kDa molecular weight. The medium was centrifuged to remove the bacteria, and sterilised by filtration through a 0.45 µm filter. The sterile filtrate was concentrated by ultrafiltration over a 10 kDa molecular weight cut-off membrane.

Proteins were isolated from the concentrated culture filtrate by precipitation with 10% trichloroacetic acid. The precipitated proteins were re-dissolved in 100 mM Tris.HCl pH 8.0. and re-precipitated by the addition of an equal volume of acetone. The acetone precipitate was dissolved in water, and proteins were re-precipitated by the addition of an equal volume of chloroform:methanol 2:1 (v/v). The chloroform:methanol precipitate was dissolved in water, and the solution was freeze-dried.

The freeze-dried protein was dissolved in iso-electric focusing buffer, containing 8 M deionised urea, 2% Triton X-100, 10 mM dithiothreitol and 2% ampholytes (pH 2.5 - 5.0). The sample was fractionated by preparative iso-electric focusing on a horizontal bed of Ultradex gel at 8 watts constant power for 16 hours. Proteins were eluted from the gel bed fractions with water and concentrated by precipitation with 10% trichloroacetic acid.

Pools of fractions containing proteins of interest were identified by analytical polyacrylamide gel electrophoresis and fractionated by preparative polyacrylamide gel electrophoresis. Samples were fractionated on 12.5% SDS-PAGE gels, and electroblotted onto nitrocellulose membranes. Proteins were located on the membranes by staining with Ponceau Red, destained with water and eluted from the membranes with 40% acetonitrile/0.1M ammonium bicarbonate pH 8.9 and then concentrated by lyophilisation.

Eluted proteins were assayed for their ability to induce proliferation and interferon- γ secretion from the peripheral blood lymphocytes of immune donors as detailed above. Proteins inducing a strong response in these assays were selected for further study.

Selected proteins were further purified by reversed-phase chromatography on a Vydac Protein C4 column, using a trifluoroacetic acid-acetonitrile system. Purified proteins were prepared for protein sequence determination by SDS-polyacrylamide gel electrophoresis, and electroblotted onto PVDF membranes. Protein sequences were determined as in Example 3. The proteins were named GV-40, GV-41, GV-42, GV-43 and GV-44. The determined N-terminal sequences for these polypeptides are shown in SEQ ID NOS: 101-105, respectively. Subsequent studies led to the isolation of a 5', middle fragment and 3' DNA sequence for GV-42 (SEQ ID NO: 136, 137 and 138, respectively). The corresponding predicted amino acid sequences are provided in SEQ ID NO: 139, 140 and 141, respectively.

Following standard DNA amplification and cloning procedures as described in Example 13, the genes encoding GV-41 and GV-42 were cloned. The determined nucleotide sequences are provided in SEQ ID NOS: 179 and 180, respectively, and the predicted amino acid sequences in SEQ ID NOS: 181 and 182. Further experiments lead to the cloning of the full-length gene encoding GV-41, which was named GV-41B. The determined nucleotide sequence and the predicted amino acid sequence of GV-41B are provided in SEQ ID NOS: 202 and 203, respectively. GV-41 had homology to the ribosome recycling factor of *M. tuberculosis* and *M. leprae*, and GV-42 had homology to a *M. avium* fibronectin attachment protein FAP-A. Within the full-length sequence of GV-42, the amino acid sequence determined for GV-43 (SEQ ID NO: 104) was identified, indicating that the amino acid sequences for GV-42 and GV-43 were obtained from the same protein.

Murine polyclonal antisera were prepared against GV-40 and GV-44 following standard procedures. These antisera were used to screen a *M. vaccae* genomic DNA library consisting of randomly sheared DNA fragments. Clones encoding GV-40 and GV-44 were identified and sequenced. The determined nucleotide sequence of the partial gene encoding GV-40 is provided in SEQ ID NO: 183 and the predicted amino acid sequence in SEQ ID NO:184. The complete gene encoding GV-40 was not cloned, and the antigen encoded by this partial gene was named GV-40P. An extended DNA sequence for GV-40P is provided in SEQ ID NO: 206 with the corresponding predicted amino acid sequence being provided in SEQ ID NO 207. The determined nucleotide sequence of the gene encoding GV-44 is provided in SEQ ID NO: 185, and the predicted amino acid sequence in SEQ ID NO: 186. With further sequencing, the determined DNA sequence for the full-length gene encoding GV-44 was obtained and is provided in SEQ ID NO 204, with the corresponding predicted amino acid sequence being provided in SEQ ID NO: 205. Homology of GV-40 to *M. leprae* Elongation factor G was found and GV-44 had homology to *M. leprae* glyceraldehyde-3-phosphate dehydrogenase.

EXAMPLE 17

ISOLATION OF THE DD-*M. VACCAE* ANTIGENS GV-45 AND GV-46

Proteins were extracted from DD-*M. vaccae* (500 mg; prepared as described above) by suspension in 10 ml 2% SDS/PBS and heating to 50 °C for 2 h. The insoluble residue was removed by centrifugation, and proteins precipitated from the supernatant by adding an equal volume of acetone and incubating at -20 °C for 1 hr. The precipitated proteins were collected by centrifugation, dissolved in reducing sample buffer, and fractionated by preparative SDS-polyacrylamide gel electrophoresis. The separated proteins were electroblotted onto PVDF membrane in 10 mM CAPS/0.01% SDS pH 11.0, and N-terminal sequences were determined in a gas-phase sequenator.

From these experiments, a protein represented by a band of approximate molecular weight of 30 kDa, designated GV-45, was isolated. The determined N-terminal sequence for GV-45 is provided in SEQ ID NO: 187. From the same experiments, a protein of

approximate molecular weight of 14 kDa, designated GV-46, was obtained. The determined N-terminal amino acid sequence of GV-46 is provided in SEQ ID NO: 208. GV-46 is homologous to the highly conserved mycobacterial host integration factor of *M. tuberculosis* and *M. smegmatis*.

From the amino acid sequence of GV-45, degenerate oligonucleotides KR32 and KR33 (SEQ ID NOS: 188 and 189, respectively) were designed. A 100 bp fragment was amplified, cloned into plasmid pBluescript II SK⁺ (Stratagene, La Jolla, CA) and sequenced (SEQ ID NO:190) following standard procedures (Sambrook, *Ibid*). The cloned insert was used to screen a *M. vaccae* genomic DNA library constructed in the *Bam*HI-site of lambda ZAP-Express (Stratagene). The isolated clone showed homology to a 35 kDa *M. tuberculosis* and a 22 kDa *M. leprae* protein containing bacterial histone-like motifs at the N-terminus and a unique C-terminus consisting of a five amino acid basic repeat. The determined nucleotide sequence for GV-45 is provided in SEQ ID NO: 191, with the corresponding predicted amino acid sequence being provided in SEQ ID NO: 192. With additional sequencing, the determined DNA sequence for the full-length gene encoding GV-45 was obtained and is provided in SEQ ID NO: 200, with the corresponding predicted amino acid sequence in SEQ ID NO: 201.

EXAMPLE 18

IMMUNOGENICITY AND IMMUNOMODULATING PROPERTIES OF RECOMBINANT PROTEINS DERIVED FROM *M. VACCAE*

A. INDUCTION OF T CELL PROLIFERATION AND IFN- γ PRODUCTION

The immunogenicity of *Mycobacterium vaccae* recombinant proteins (GV recombinant proteins) was tested by injecting female BALB/cByJ mice in each hind foot-pad with 10 ug of recombinant GV proteins emulsified in incomplete Freund's adjuvant (IFA). Control mice received phosphate buffered saline in IFA. The draining popliteal lymph nodes were excised 10 days later and the cells obtained therefrom were stimulated with the immunizing GV protein and assayed for proliferation by measuring the uptake of tritiated

thymidine. The amount of interferon gamma (IFN γ) produced and secreted by these cells into the culture supernatants was assayed by standard enzyme-linked immunoassay.

As shown in Table 21 summarising proliferative responses, all GV proteins were found to induce a T cell proliferative response. The lymph node T cells from an immunized mouse proliferated in response to the specific GV protein used in the immunization. Lymph node cells from non-immunised mice did not proliferate in response to GV proteins. The data in Table 22 showing IFN γ production, indicate that most of the GV proteins stimulated IFN γ production by lymph node cells from mice immunised with the corresponding GV protein. When lymph node cells from non-immunized mice were cultured with individual GV proteins, IFN γ production was not detectable.

The GV proteins are thus immunogenic in being able to stimulate T cell proliferation and/or IFN γ production when administered by subcutaneous injection. The antigen-specific stimulatory effects on T cell proliferation and IFN γ production are two advantageous properties of candidate vaccines for tuberculosis.

TABLE 21
Immunogenic Properties of GV proteins: Proliferation

GV protein	Proliferation (cpm)		
	Dose of GV protein used <i>in vitro</i> (µg/ml)		
	50	2	0.08
GV-1/70	31,550 ± 803	19,058 ± 2,449	5,596 ± 686
GV-1/83	18,549 ± 2,716	23,932 ± 1,964	11,787 ± 1,128
GV-3	34,751 ± 1,382	6,379 ± 319	4,590 ± 1,042
GV-4P	26,460 ± 1,877	10,370 ± 667	6,685 ± 673
GV-5	42,418 ± 2,444	23,902 ± 2,312	13,973 ± 772
GV-5P	35,691 ± 159	14,457 ± 1,185	8,340 ± 725
GV-7	38,686 ± 974	22,074 ± 3,698	15,906 ± 1,687
GV-9	30,599 ± 2580	15,260 ± 2,764	4,531 ± 1,240
GV-13	15,296 ± 2,006	7,163 ± 833	3,701 ± 243
GV-14	27,754 ± 1,872	13,001 ± 3,273	9,897 ± 2,833
GV-14B	10,761 ± 485	5,075 ± 1,470	2,341 ± 289
GV-22B	3,199 ± 771	3,255 ± 386	1,841 ± 318
GV-23	35,598 ± 1,330	15,423 ± 2,858	7,393 ± 2,188
GV-24B	43,678 ± 2,190	30,307 ± 1,533	15,375 ± 2,594
GV-27	18,165 ± 3,300	16,329 ± 1,794	6,107 ± 1,773
GV-27A	23,723 ± 850	6,860 ± 746	4,295 ± 780
GV-27B	31,602 ± 1,939	29,468 ± 3,867	30,306 ± 1,912
GV-29	20,034 ± 3,328	8,107 ± 488	2,982 ± 897
GV-33	41,529 ± 1,919	27,529 ± 1,238	8,764 ± 256
GV-35	29,163 ± 2,693	9,968 ± 314	1,626 ± 406
GV-38AP	28,971 ± 4,499	17,396 ± 878	8,060 ± 810
GV-38BP	19,746 ± 245	11,732 ± 3,207	6,264 ± 875
GV-40P	25,185 ± 2,877	19,292 ± 2,294	10,883 ± 893
GV-41B	24,646 ± 2,714	12,627 ± 3,622	5,772 ± 1,041
GV-42	25,486 ± 3,029	20,591 ± 2,021	13,789 ± 775
GV-44	2,684 ± 1,995	3,577 ± 1,725	1,499 ± 959
GV-45	9,554 ± 482	3,683 ± 1,127	1,497 ± 199

TABLE 22
Immunogenic properties of GV proteins: IFN γ production

GV protein	IFN γ (ng/ml)		
	Dose of GV protein used <i>in vitro</i> (μ g/ml)		
	50	10	2
GV-1/70	24.39 \pm 6.66	6.19 \pm 1.42	1.90 \pm 0.53
GV-1/83	11.34 \pm 5.46	5.36 \pm 1.34	2.73 \pm 1.55
GV-3	3.46 \pm 0.30	1.57 \pm 0.04	not detectable
GV-4P	6.48 \pm 0.37	3.00 \pm 0.52	1.38 \pm 0.50
GV-5	4.08 \pm 1.41	6.10 \pm 2.72	2.35 \pm 0.40
GV-5P	34.98 \pm 15.26	9.95 \pm 3.42	5.68 \pm 0.79
GV-7	33.52 \pm 3.08	25.47 \pm 4.14	9.60 \pm 1.74
GV-9	92.27 \pm 45.50	88.54 \pm 16.48	30.46 \pm 1.77
GV-13	11.60 \pm 2.89	2.04 \pm 0.58	1.46 \pm 0.62
GV-14	8.28 \pm 1.56	3.19 \pm 0.56	0.94 \pm 0.24
GV-14B	not detectable	not detectable	not detectable
GV-22B	not detectable	not detectable	not detectable
GV-23	59.67 \pm 14.88	30.70 \pm 4.48	9.17 \pm 1.51
GV-24B	6.76 \pm 0.58	3.20 \pm 0.50	1.97 \pm 0.03
GV-27	72.22 \pm 11.14	30.86 \pm 10.55	21.38 \pm 3.12
GV-27A	4.25 \pm 2.32	1.51 \pm 0.73	not detectable
GV-27B	87.98 \pm 15.78	44.43 \pm 8.70	21.49 \pm 5.60
GV-29	7.56 \pm 2.58	1.22 \pm 0.56	not detectable
GV-33	7.71 \pm 0.26	8.44 \pm 2.35	1.52 \pm 0.24
GV-38AP	23.49 \pm 5.89	8.87 \pm 1.62	4.17 \pm 1.72
GV-38BP	5.30 \pm 0.95	3.10 \pm 1.19	1.91 \pm 1.01
GV-40P	15.65 \pm 7.89	10.58 \pm 1.31	3.57 \pm 1.53
GV-41B	16.73 \pm 1.61	5.08 \pm 1.08	2.13 \pm 1.10
GV-42	95.97 \pm 23.86	52.88 \pm 5.79	30.06 \pm 8.94
GV-44	not detectable	not detectable	not detectable

B. ACTIVATION OF LYMPHOCYTE SUBPOPULATIONS

The ability of recombinant *M. vaccae* proteins of the present invention, heat-killed *M. vaccae* and DD-*M. vaccae* to activate lymphocyte subpopulations was determined by examining upregulation of expression of CD69 (a surface protein expressed on activated cells).

PBMC from normal donors (5×10^6 cells/ml) were stimulated with 20 ug/ml of either heat-killed *M. vaccae* cells, DD-*M. vaccae* or recombinant GV-22B (SEQ ID NO: 145), GV-23 (SEQ ID NO: 89), GV-27 (SEQ ID NO: 160), GV27A (SEQ ID NO: 117), GV-27B (SEQ ID NO: 162) or GV-45 (SEQ ID NO: 201) for 24 hours. CD69 expression was determined by staining cultured cells with monoclonal antibody against CD56, $\alpha\beta$ T cells or $\gamma\delta$ T cells, in combination with monoclonal antibodies against CD69, followed by flow cytometry analysis

Table 23 shows the percentage of $\alpha\beta$ T cells, $\gamma\delta$ T cells and NK cells expressing CD69 following stimulation with heat-killed *M. vaccae*, DD-*M. vaccae* or recombinant *M. vaccae* proteins. These results demonstrate that heat-killed *M. vaccae*, DD-*M. vaccae* and GV-23 stimulate the expression of CD69 in the lymphocyte subpopulations tested compared with control (non-stimulated cells), with particularly high levels of CD69 expression being seen in NK cells. GV-45 was found to upregulate CD69 expression in $\alpha\beta$ T cells.

TABLE 23
Stimulation of CD69 Expression

	$\alpha\beta$ T cells	$\gamma\delta$ T cells	NK cells
Control	3.8	6.2	4.8
Heat-killed <i>M. vaccae</i>	8.3	10.2	40.3
DD- <i>M. vaccae</i>	10.1	17.5	49.9
GV-22B	5.6	3.9	8.6
GV-23	5.8	10.0	46.8
GV-27	5.5	4.4	13.3
GV-27A	5.5	4.4	13.3
GV-27B	4.4	2.8	7.1
GV-45	11.7	4.9	6.3

The ability of the recombinant protein GV-23 (20 ug/ml) to induce CD69 expression in lymphocyte subpopulations was compared with that of the known Th1-inducing adjuvants MPL/TDM/CWS (Monophosphoryl Lipid A/ Trehalose 6'6' dimycolate; Sigma, St. Louis, MO; at a final dilution of 1:20) and CpG ODN (Promega, Madison, WI; 20 ug/ml), and the known Th2-inducing adjuvants aluminium hydroxide (Superfos Biosector, Kvistgard, Denmark; at a final dilution of 1:400) and cholera toxin (20 ug/ml), using the procedure described above. MPL/TDM/CWS and aluminium hydroxide were employed at the maximum concentration that does not cause cell cytotoxicity. Figs. 8A-C show the stimulation of CD69 expression on $\alpha\beta$ T cells, $\gamma\delta$ T cells and NK cells, respectively. GV-23, MPL/TDM/CWS and CpG ODN induced CD69 expression on NK cells, whereas aluminium hydroxide and cholera toxin did not.

C. STIMULATION OF CYTOKINE PRODUCTION

The ability of recombinant *M. vaccae* proteins of the present invention to stimulate cytokine production in PBMC was examined as follows. PBMC from normal donors (5×10^6 cells/ml) were stimulated with 20 ug/ml of either heat-killed *M. vaccae* cells, DD-*M. vaccae*, or recombinant GV-22B (SEQ ID NO: 145), GV-23 (SEQ ID NO: 89), GV-27 (SEQ ID NO: 160), GV27A (SEQ ID NO: 117), GV-27B (SEQ ID NO: 162) or GV-45 (SEQ ID NO: 201) for 24 hours. Culture supernatants were harvested and tested for the production of IL-1 β , TNF- α , IL-12 and IFN- γ using standard ELISA kits (Genzyme, Cambridge, MA), following the manufacturer's instructions. Figs. 9A-D show the stimulation of IL-1 β , TNF- α , IL-12 and IFN- γ production, respectively. Heat-killed *M. vaccae* and DD-*M. vaccae* were found to stimulate the production of all four cytokines examined, while recombinant GV-23 and GV-45 were found to stimulate the production of IL-1 β , TNF- α and IL-12. Figs. 10A-C show the stimulation of IL-1 β , TNF- α and IL-12 production, respectively, in human PBMC (determined as described above) by varying concentrations of GV-23 and GV-45.

Figs. 11A-D show the stimulation of IL-1 β , TNF- α , IL-12 and IFN- γ production, respectively, in PBMC by GV-23 as compared to that by the adjuvants MPL/TDM/CWS (at a final dilution of 1:20), CpG ODN (20 ug/ml), aluminium hydroxide (at a final dilution of 1:400) and cholera toxin (20 ug/ml). GV-23, MPL/TDM/CWS and CpG ODN induced significant levels of the four cytokines examined, with higher levels of IL-1 β production being seen with GV-23 than with any of the known adjuvants. Aluminium hydroxide and cholera toxin induced only negligible amounts of the four cytokines.

D. ACTIVATION OF ANTIGEN PRESENTING CELLS

The ability of heat-killed *M. vaccae*, DD-*M. vaccae* and recombinant *M. vaccae* proteins to enhance the expression of the co-stimulatory molecules CD40, CD80 and CD86 on B cells, monocytes and dendritic cells was examined as follows.

Peripheral blood mononuclear cells depleted of T cells and comprising mainly B cells, monocytes and dendritic cells were stimulated with 20 ug/ml of either heat-killed *M. vaccae* cells, DD-*M. vaccae*, or recombinant GV-22B (SEQ ID NO: 145), GV-23 (SEQ ID NO: 89),

GV-27 (SEQ ID NO: 160), GV27A (SEQ ID NO: 117), GV-27B (SEQ ID NO: 162) or GV-45 (SEQ ID NO: 201) for 48 hours. Stimulated cells were harvested and analyzed for up-regulation of CD40, CD80 and CD86 using 3 color flow cytometric analysis. Tables 24, 25 and 26 show the fold increase in mean fluorescence intensity from control (non-stimulated cells) for dendritic cells, monocytes, and B cells, respectively.

TABLE 24

Stimulation of CD40, CD80 and CD86 Expression on Dendritic Cells

	CD40	CD80	CD86
Control	0	0	0
Heat-killed <i>M. vaccae</i>	6.1	3.8	1.6
DD- <i>M. vaccae</i>	6.6	4.2	1.6
GV-22B	4.6	1.9	1.6
GV-23	6.0	4.5	1.8
GV-27	5.2	1.9	1.6
GV-27A	2.3	0.9	1.0
GV-27B	2.6	1.1	1.1
GV-45	5.8	3.0	3.1

TABLE 25

Stimulation of CD40, CD80 and CD86 Expression on Monocytes

	CD40	CD80	CD86
Control	0	0	0
Heat-killed <i>M. vaccae</i>	2.3	1.8	0.7
DD- <i>M. vaccae</i>	1.9	1.5	0.7
GV-22B	0.7	0.9	1.1
GV-23	2.3	1.5	0.7
GV-27	1.5	1.4	1.2
GV-27A	1.4	1.4	1.4
GV-27B	1.6	1.2	1.2
GV-45	1.6	1.2	1.0

TABLE 26

Stimulation of CD40, CD80 and CD86 Expression on B Cells

	CD40	CD80	CD86
Control	0	0	0
Heat-killed <i>M. vaccae</i>	1.6	1.0	1.7
DD- <i>M. vaccae</i>	1.5	0.9	1.7
GV-22B	1.1	0.9	1.2
GV-23	1.2	1.1	1.4
GV-27	1.1	0.9	1.1
GV-27A	1.0	1.1	0.9
GV-27B	1.0	0.9	0.9
GV-45	1.2	1.1	1.3

As shown above, increased levels of CD40, CD80 and CD86 expression were seen in dendritic cells, monocytes and B cells with all the compositions tested. Expression levels were most increased in dendritic cells, with the highest levels of expression being obtained with heat-killed *M. vaccae*, DD-*M. vaccae*, GV-23 and GV-45. Figs. 12A-C show the stimulation of expression of CD40, CD80 and CD86, respectively, in dendritic cells by varying concentrations of GV-23 and GV-45.

The ability of GV-23 to stimulate CD40, CD80 and CD86 expression in dendritic cells was compared to that of the Th1-inducing adjuvants MPL/TDM/CWS (at a final dilution of 1:20) and CpG ODN (20 ug/ml), and the known Th2-inducing adjuvants aluminium hydroxide (at a final dilution of 1:400) and cholera toxin (20 ug/ml). GV23, MPL/TDM/CWS and CpG ODN caused significant up-regulation of CD40, CD80 and CD86, whereas cholera toxin and aluminium hydroxide induced modest or negligible dendritic cell activation, respectively.

E. DENDRITIC CELL MATURATION AND FUNCTION

The effect of the recombinant *M. vaccae* protein GV-23 on the maturation and function of dendritic cells was examined as follows.

Purified dendritic cells ($5 \times 10^4 - 10^5$ cells/ml) were stimulated with GV-23 (20 ug/ml) or LPS (10 ug/ml) as a positive control. Cells were cultured for 20 hour and then analyzed for CD83 (a maturation marker) and CD80 expression by flow cytometry. Non-stimulated cells were used as a negative control. The results are shown below in Table 27.

TABLE 27
Stimulation of CD83 Expression in Dendritic Cells

Treatments	%CD83-positive dendritic cells	% CD80-positive dendritic cells
Control	15 \pm 8	9 \pm 6.6
GV-23	35 \pm 13.2	24.7 \pm 14.2
LPS	36.3 \pm 14.8	27.7 \pm 13

Data = mean \pm SD (n=3)

The ability of GV-23 to enhance dendritic cell function as antigen presenting cells was determined by mixed lymphocyte reaction (MLR) assay. Purified dendritic cells were culture in medium alone or with GV-23 (20 ug/ml) for 18-20 hours and then stimulated with allogeneic T cells (2×10^5 cells/well). After 3 days of incubation, (^3H)-thymidine was added. Cells were harvested 1 day later and the uptake of radioactivity was measured. Fig. 13 shows the increase in uptake of (^3H)-thymidine with increase in the ratio of dendritic cells to T cells. Significantly higher levels of radioactivity uptake were seen in GV-23 stimulated dendritic cells compared to non-stimulated cells, showing that GV-23 enhances dendritic cell mixed leukocyte reaction.

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, changes and modifications can be carried out without departing from the scope of the invention which is intended to be limited only by the scope of the appended claims.

Claims

1. A polypeptide comprising an immunogenic portion of an isolated *M. vaccae* antigen, wherein the antigen includes a sequence selected from the group consisting of: sequences recited in SEQ ID NOS: 143, 145, 147, 152, 154 156, 158, 160, 162, 165, 166, 170, 172, 174, 177, 178, 181, 182, 184, 186, 187, 192, 194, 196, 197, 199, 201, 203, 205 and 207.

2. A polypeptide comprising an immunogenic portion of an isolated *M. vaccae* antigen, wherein the antigen includes a sequence selected from the group consisting of:

- (a) sequences having at least about 50% identical residues to a sequence recited in SEQ ID NOS: 143, 145, 147, 152, 154 156, 158, 160, 162, 165, 166, 170, 172, 174, 177, 178, 181, 182, 184, 186, 187, 192, 194, 196, 197, 199, 201, 203, 205 and 207 as measured by computer algorithm BLASTP;
- (b) sequences having at least about 75% identical residues to a sequence recited in SEQ ID NOS: 143, 145, 147, 152, 154 156, 158, 160, 162, 165, 166, 170, 172, 174, 177, 178, 181, 182, 184, 186, 187, 192, 194, 196, 197, 199, 201, 203, 205 and 207 as measured by computer algorithm BLASTP; and
- (c) sequences having at least about 95% identical residues to a sequence recited in SEQ ID NOS: 143, 145, 147, 152, 154 156, 158, 160, 162, 165, 166, 170, 172, 174, 177, 178, 181, 182, 184, 186, 187, 192, 194, 196, 197, 199, 201, 203, 205 and 207 as measured by computer algorithm BLASTP.

3. A polypeptide comprising an immunogenic portion of an isolated *M. vaccae* antigen, wherein the antigen comprises an amino acid sequence encoded by a polynucleotide selected from the group consisting of:

- (a) sequences recited in SEQ ID NOS: 142, 144, 146, 151, 153, 155, 157, 159, 161, 163, 164, 169, 171, 173, 175, 176, 179, 180, 183, 185, 191, 193, 195, 198 and 200;
- (b) complements of the sequences recited in SEQ ID NOS: 142, 144, 146, 151, 153, 155, 157, 159, 161, 163, 164, 169, 171, 173, 175, 176, 179, 180, 183, 185, 191, 193, 195, 198 and 200; and

- (c) sequences having at least about a 99% probability of being the same as a sequence of (a) or (b) as measured by computer algorithm BLASTN.
4. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide according to any one of claims 1-3.
5. An expression vector comprising a polynucleotide according to claim 4.
6. A host cell transformed with an expression vector according to claim 5.
7. The host cell of claim 6, wherein the host cell is selected from the group consisting of *E. coli*, mycobacteria, insect, yeast and mammalian cells.
8. A fusion protein comprising at least one polypeptide according to any one of claims 1-3.
9. A pharmaceutical composition comprising a polypeptide according to any one of claims 1-3 and a physiologically acceptable carrier.
10. A pharmaceutical composition comprising a polynucleotide according to claim 4 and a physiologically acceptable carrier.
11. A pharmaceutical composition comprising a fusion protein according to claim 8 and a physiologically acceptable carrier.
12. A vaccine comprising a polypeptide according to any one of claims 1-3 and a non-specific immune response amplifier.
13. A vaccine comprising a polynucleotide according to claim 4 and a non-specific immune response amplifier.
14. A vaccine comprising a fusion protein according to claim 8 and a non-specific immune response amplifier.
15. A vaccine according to any one of claims 12-14 wherein the non-specific immune response amplifier is an adjuvant.
16. A vaccine according to any one of claims 12-14 wherein the non-specific immune response amplifier is selected from the group consisting of:
- (a) delipidated and deglycolipidated *M. vaccae* cells;
 - (b) inactivated *M. vaccae* cells; and
 - (c) *M. vaccae* culture filtrate.

17. A method for enhancing an immune response in a patient, comprising administering to a patient a pharmaceutical composition according to any one of claims 9-11.

18. A method for enhancing an immune response in a patient, comprising administering to a patient a vaccine according to any one of claims 12-14.

19. The method of any one of claims 17 and 18, wherein the immune response is a Th1 response.

20. A method for the treatment of a disorder in a patient, comprising administering to the patient a pharmaceutical composition according to any one of claims 9-11.

21. A method for the treatment of a disorder in a patient, comprising administering to the patient a vaccine according to any one of claims 12-14.

22. The method of any one of claims 20 and 21, wherein the disorder is selected from the group consisting of immune disorders, infectious diseases, skin diseases and diseases of the respiratory system.

23. The method of claim 23 wherein the disorder is selected from the group consisting of mycobacterial infections, asthma, and psoriasis.

24. A method for the treatment of a disorder in a patient comprising administering a composition comprising a component selected from the group consisting of:

- (a) inactivated *M. vaccae* cells;
- (b) delipidated and deglycolipidated *M. vaccae* cells;
- (c) delipidated and deglycolipidated *M. vaccae* cells depleted of mycolic acids;
- (d) delipidated and deglycolipidated *M. vaccae* cells depleted of mycolic acids and arabinogalactan; and
- (e) *M. vaccae* culture filtrate,

the disorder being selected from the group consisting of immune disorders, infectious diseases, skin diseases and diseases of the respiratory system.

25. The method of claim 24, wherein the disorder is selected from the group consisting of mycobacterial infections, asthma and psoriasis.

26. A method for enhancing a non-specific immune response to an antigen comprising administering a polypeptide, the polypeptide comprising an immunogenic portion of a *M. vaccae* antigen, wherein the *M. vaccae* antigen includes a sequence selected from the group consisting of:

- (a) sequences recited in SEQ ID NO: 89 and 201; and
- (b) sequences having at least about 80% identical residues to a sequence recited in SEQ ID NO: 89 and 201 as determined by computer algorithm BLASTP.

27. A method for detecting mycobacterial infection in a patient, comprising:

- (a) contacting dermal cells of a patient with one or more polypeptides according to any one of claims 1-3; and
- (b) detecting an immune response on the patient's skin.

28. The method of claim 27 wherein the immune response is induration.

29. A diagnostic kit comprising:

- (a) a polypeptide according to any one of claims 1-3; and
- (b) apparatus sufficient to contact the polypeptide with the dermal cells of a patient.

30. A method for detecting mycobacterial infection in a biological sample, comprising:

- (a) contacting the biological sample with a polypeptide according to any one of claims 1-3; and
- (b) detecting in the sample the presence of antibodies that bind to the polypeptide.

31. The method of claim 30 wherein the polypeptide(s) are bound to a solid support.

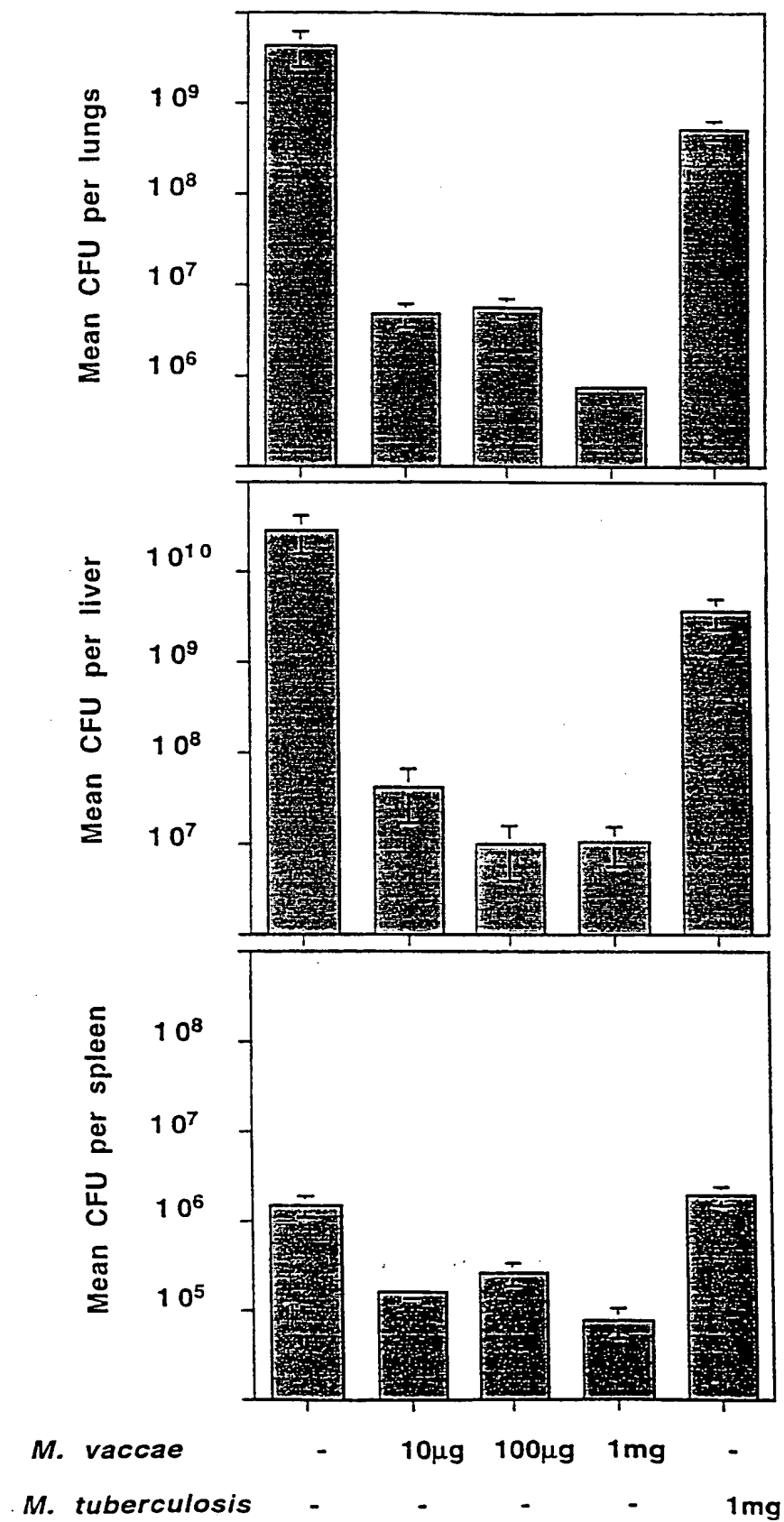
32. The method of claim 30 wherein the biological sample is selected from the group consisting of whole blood, serum, plasma, saliva, cerebrospinal fluid and urine.

33. A method for detecting mycobacterial infection in a biological sample, comprising:

- (a) contacting the biological sample with a binding agent which is capable of binding to a polypeptide according to any one of claims 1-3; and

- (b) detecting in the sample a protein or polypeptide that binds to the binding agent.
- 34. The method of claim 33 wherein the binding agent is a monoclonal antibody.
- 35. The method of claim 33 wherein the binding agent is a polyclonal antibody.
- 36. A diagnostic kit comprising:
 - (a) at least one polypeptide according to any one of claims 1-3; and
 - (b) a detection reagent.
- 37. The kit of claim 36 wherein the polypeptide is immobilized on a solid support.
- 38. The kit of claim 36 wherein the detection reagent comprises a reporter group conjugated to a binding agent.
- 39. The kit of claim 38 wherein the binding agent is selected from the group consisting of anti-immunoglobulins, Protein G, Protein A and lectins.
- 40. The kit of claim 38 wherein the reporter group is selected from the group consisting of radioisotopes, fluorescent groups, luminescent groups, enzymes, biotin and dye particles.
- 41. A monoclonal antibody that binds to a polypeptide according to any one of claims 1-3.
- 42. A polyclonal antibody that binds to a polypeptide according to any one of claims 1-3.
- 43. A method for enhancing a non-specific immune response to an antigen comprising administering a composition comprising a component selected from the group consisting of:
 - (a) delipidated and deglycolipidated *M.vaccae* cells depleted of mycolic acids; and
 - (b) delipidated and deglycolipidated *M.vaccae* cells depleted of mycolic acids and arabinogalactan.

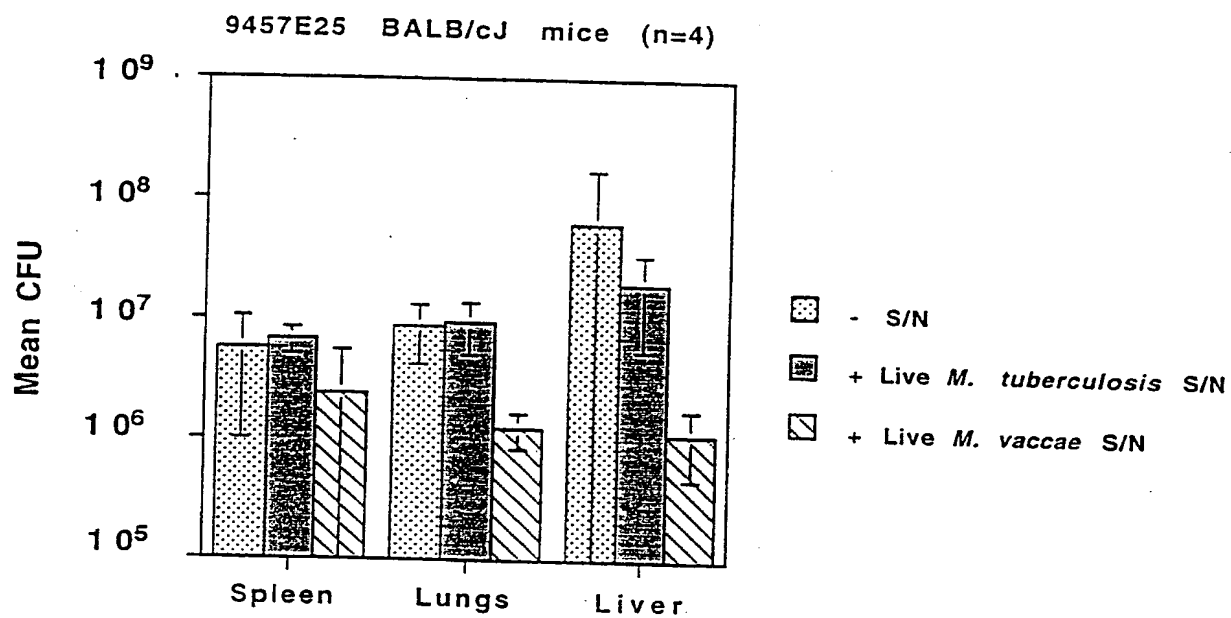
Figure 1A



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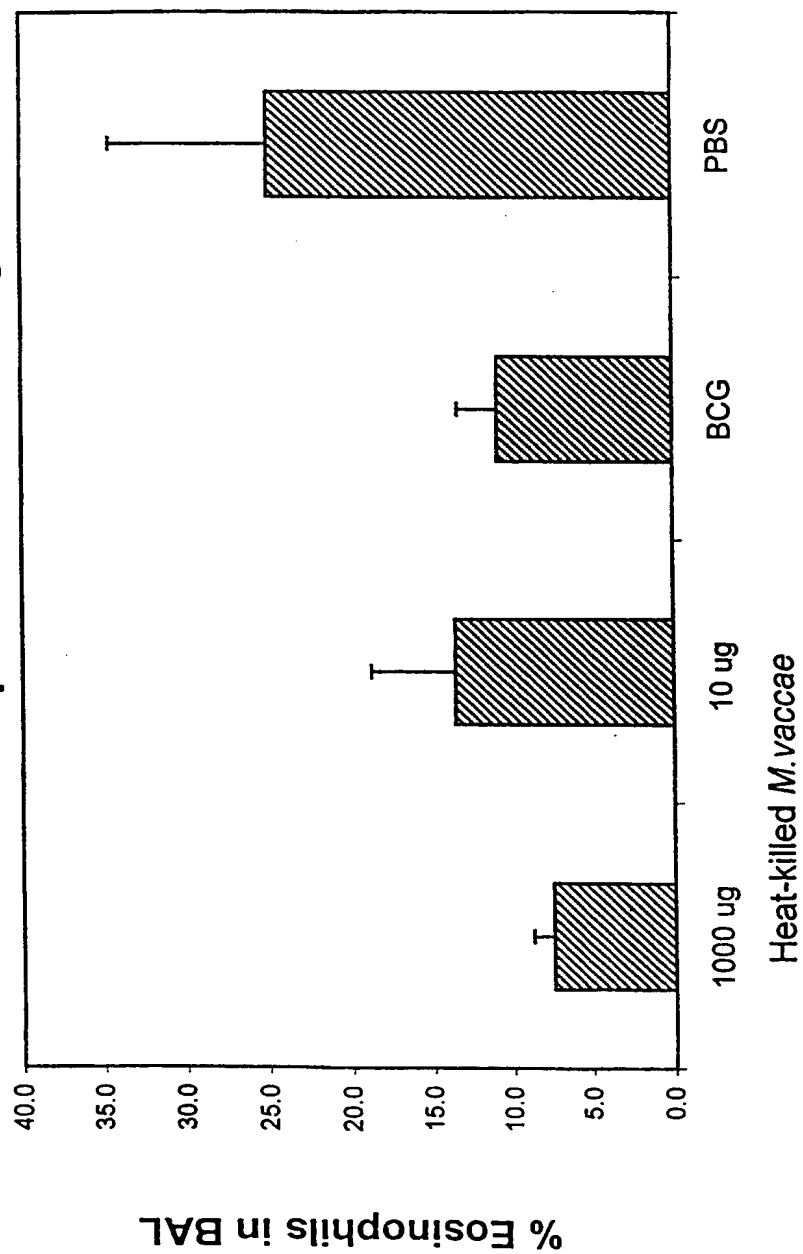
Figure 1B

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EFFECT OF IMMUNISATION WITH *M. VACCAE* CULTURE FILTRATE

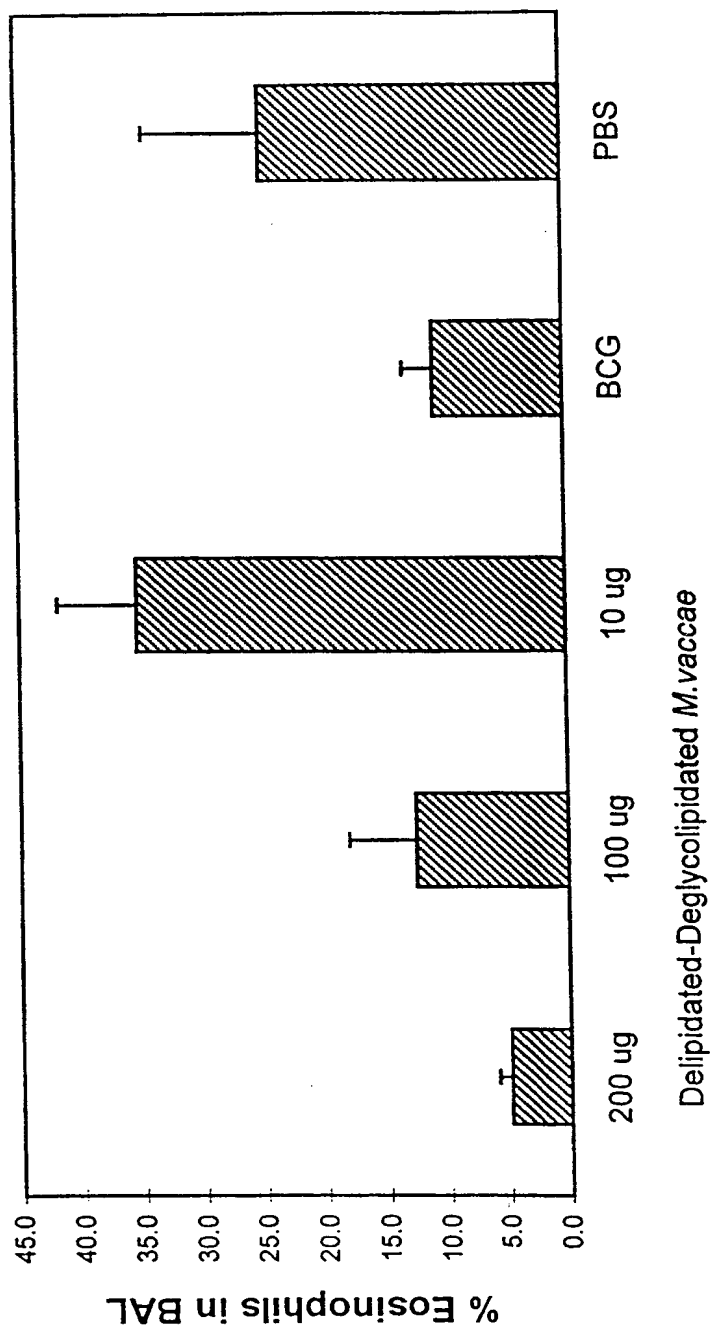
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Figure 2A
Mice treated with heat-killed *M. vaccae*
4 weeks prior to OVA challenge



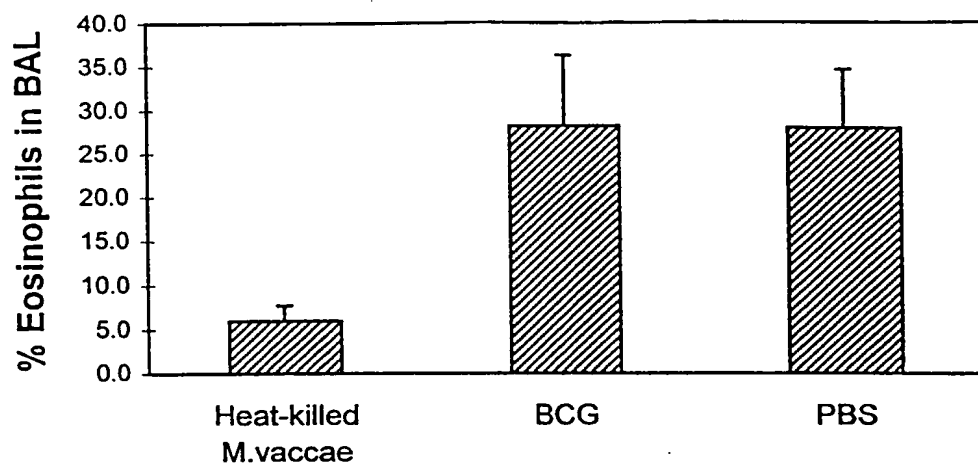
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Figure 2B
Mice treated with Delipidated-Deglycolipidated *M. vaccae*
4 weeks prior to OVA challenge



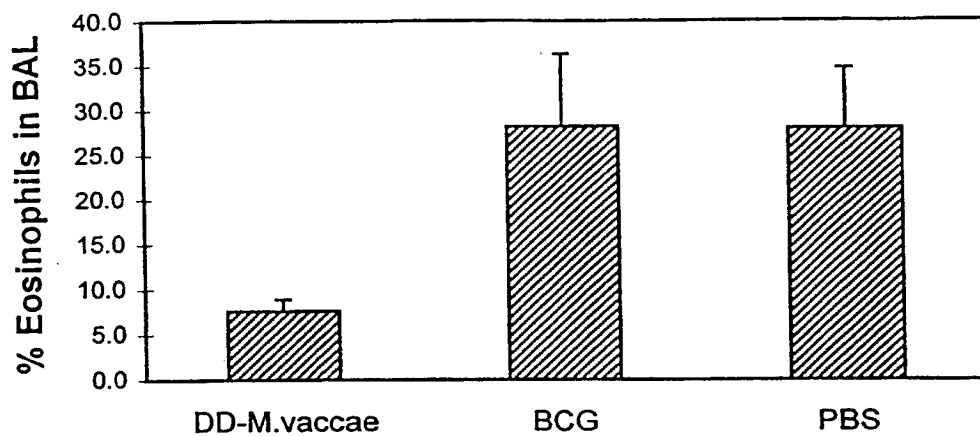
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Figure 2C
Mice treated with 1000 ug heat-killed *M.vaccae*
one week prior to OVA challenge



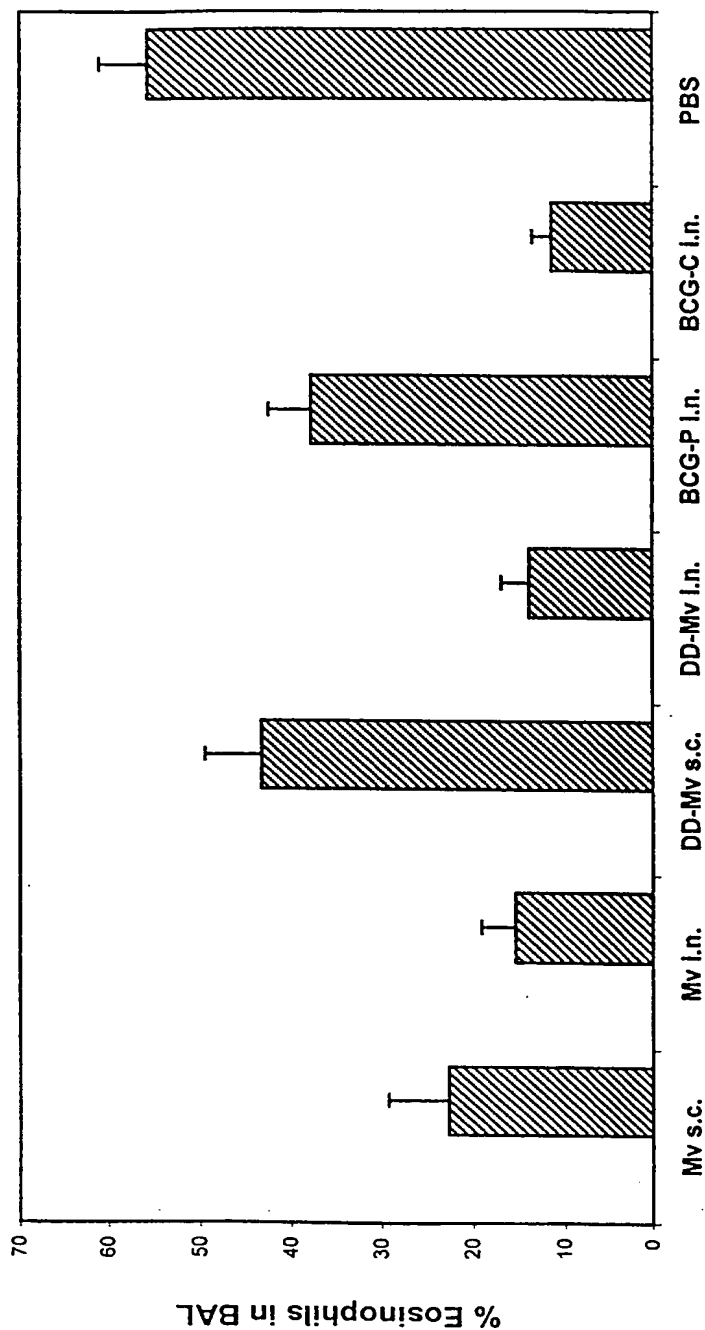
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Figure 2D
Mice treated with 200 μ g DD- *M.vaccae*
one week prior to OVA challenge



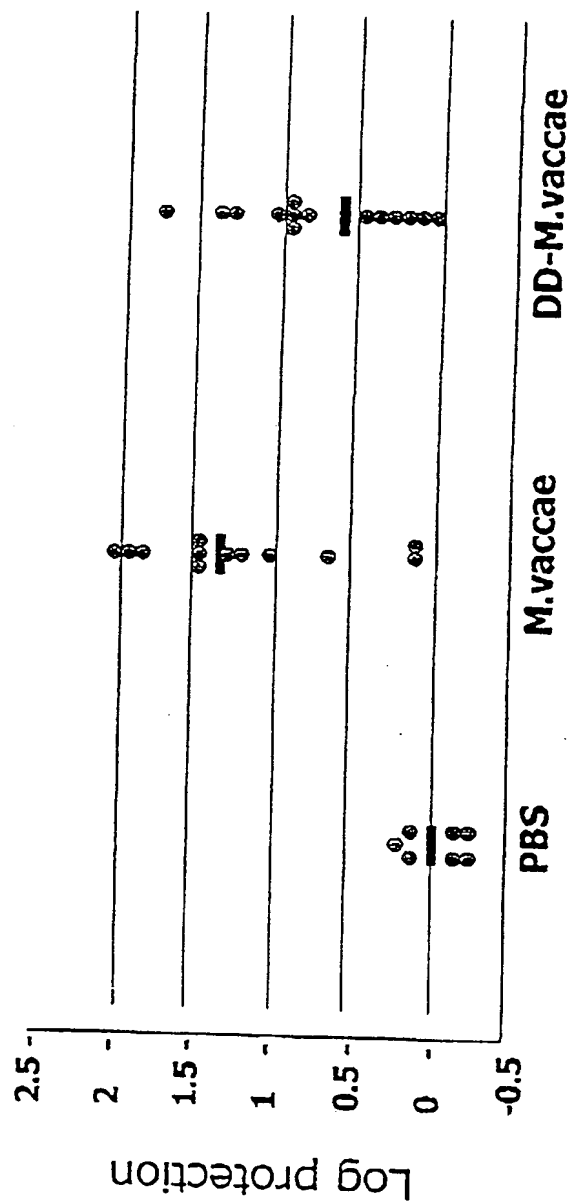
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Figure 2E
Mice treated intranasally (i.n.) or subcutaneously (s.c.)



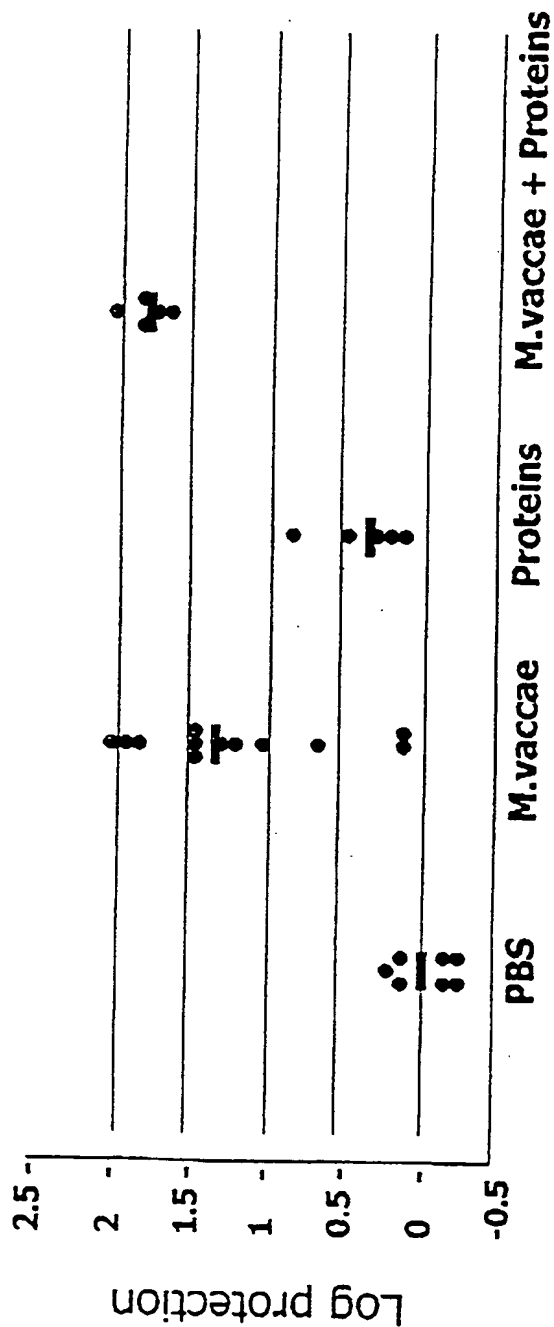
Key
Mv = *M.vaccae* DD-Mv = Delipidated deglycolipidated *M.vaccae*
BCG-P = Pasteur BCG-C = Connought

Figure 3A
**Effect of Immunizing Mice with *M.vaccae*,
and Delipidated, deglycolipidated *M.vaccae*
on Tuberculosis**



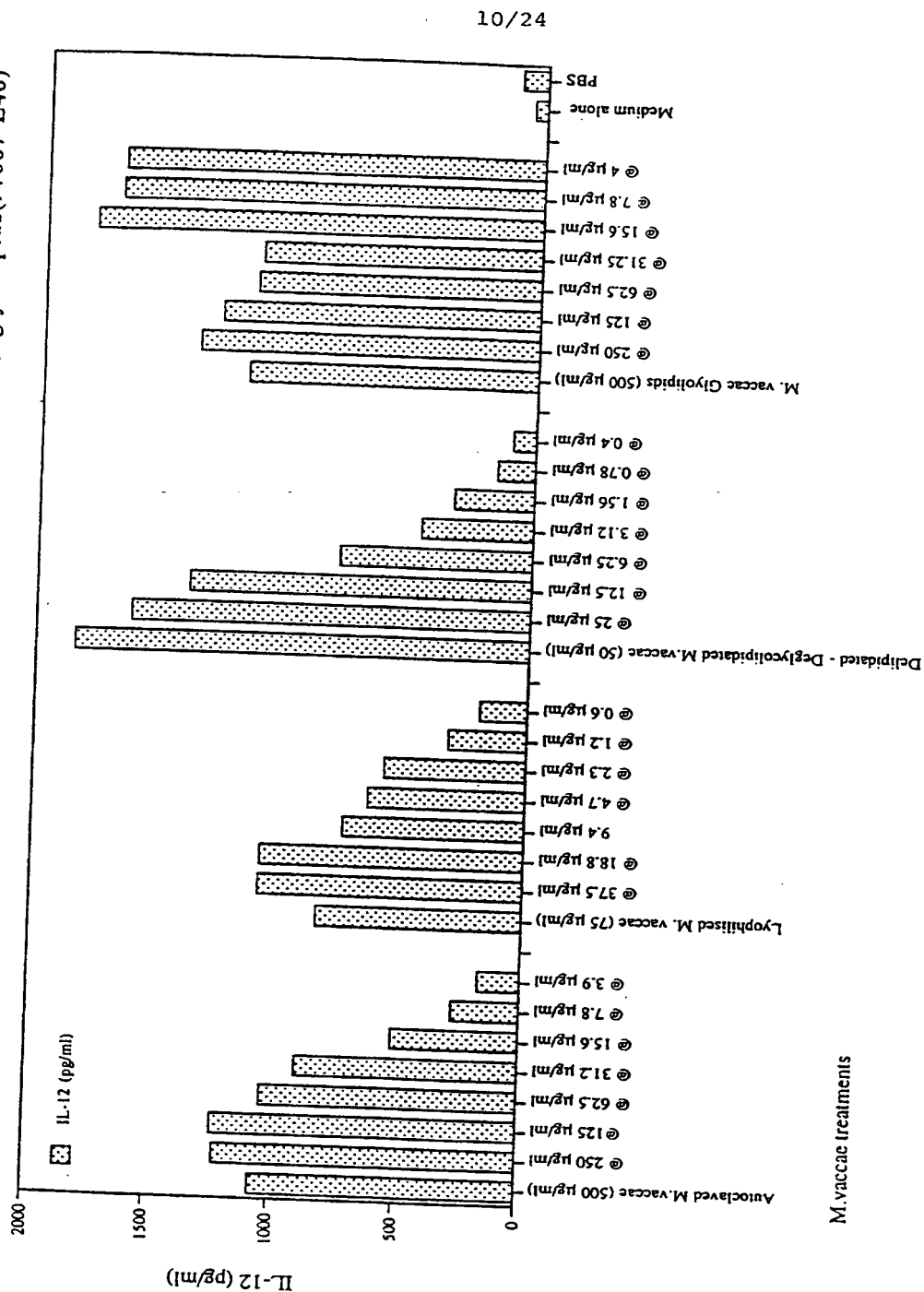
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Figure 3B
**Effect of Immunizing Mice with *M.vaccae*,
 and recombinant *M.vaccae* proteins
 on Tuberculosis**



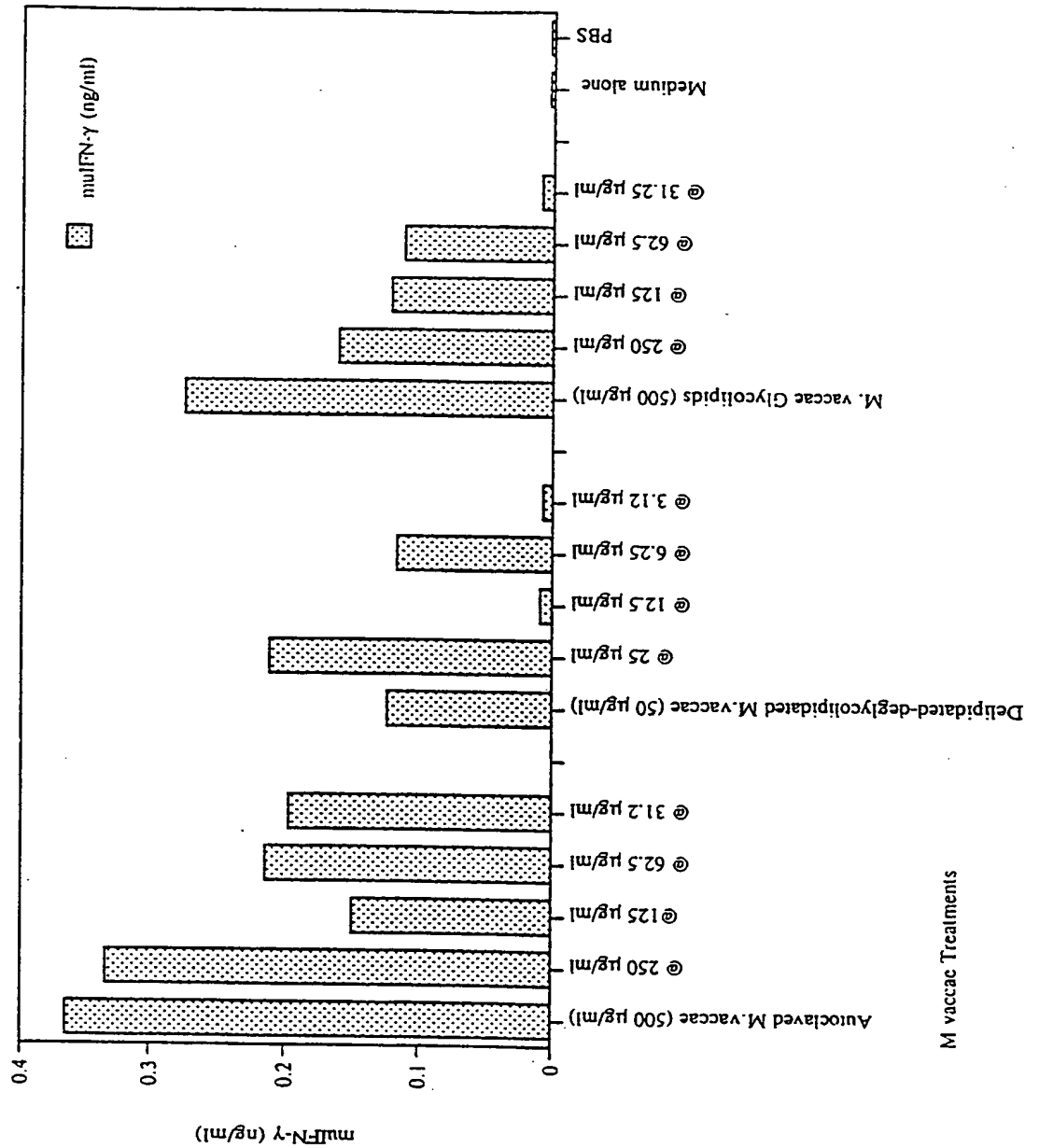
Proteins: Pool containing 15 ug each of GV 4P, 7, 9, 27B, 33.

Figure 4 Induction of IL-12 from macrophages stimulated with autoclaved *M. vaccae*, lyophilised *M. vaccae*, delipidated-deglycolipidated *M. vaccae*, or *M. vaccae* glycolipids(97067-E46)



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Figure 5 Induction of IFN- γ from SCID splenocytes stimulated with Autoclaved *M. vaccae*, Delipidated-deglycolipidated *M. vaccae* or *M. vaccae* Glycolipids (97087-E07)



M. vaccae Treatments

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Figure 6A IL-12 dose-responses from C57BL-6 peritoneal macrophages stimulated with purified GV proteins (97087-E04)

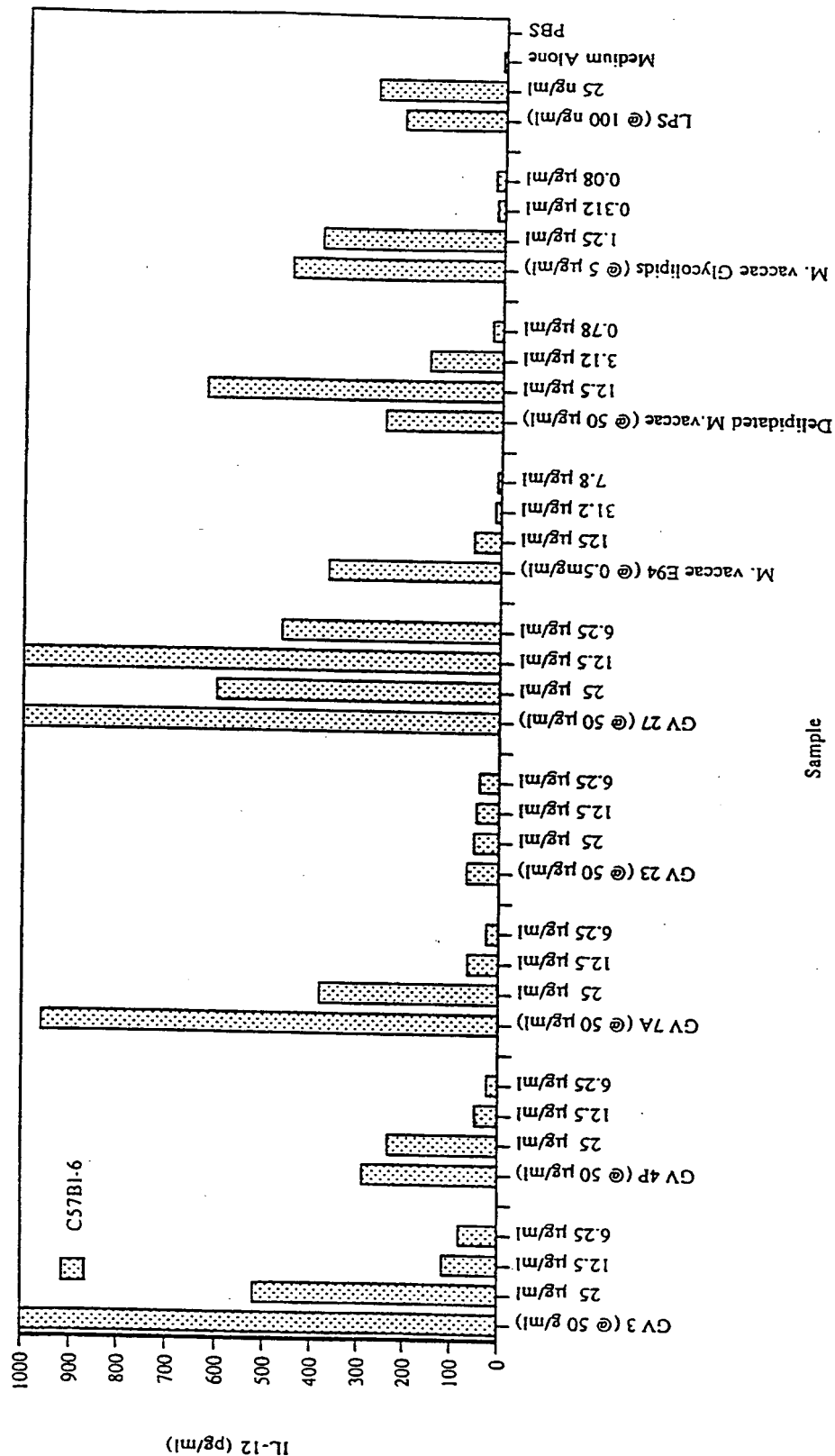


Figure 6B IL-12 dose-responses from BALB/C peritoneal macrophages stimulated with purified GV proteins (97087-E04)

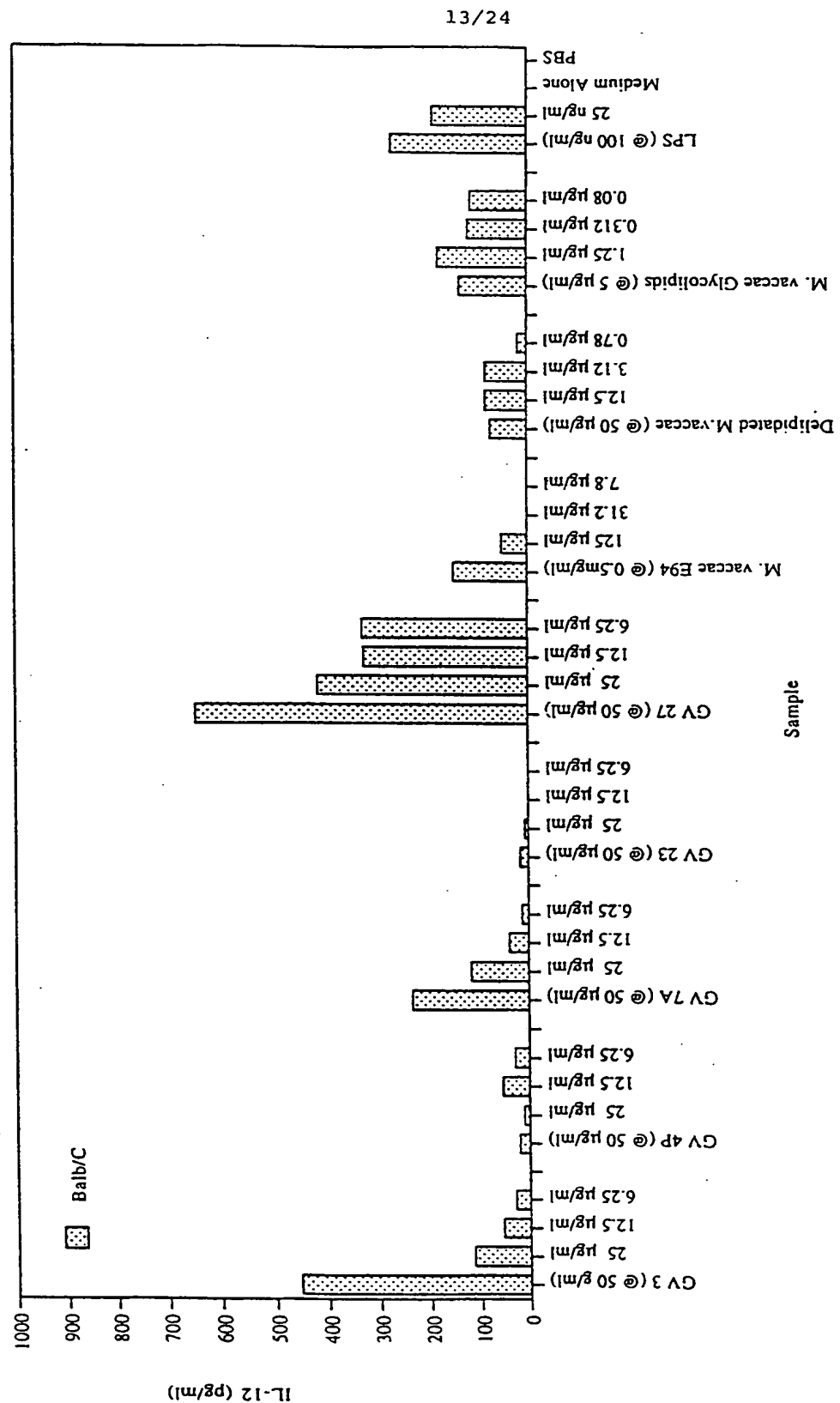
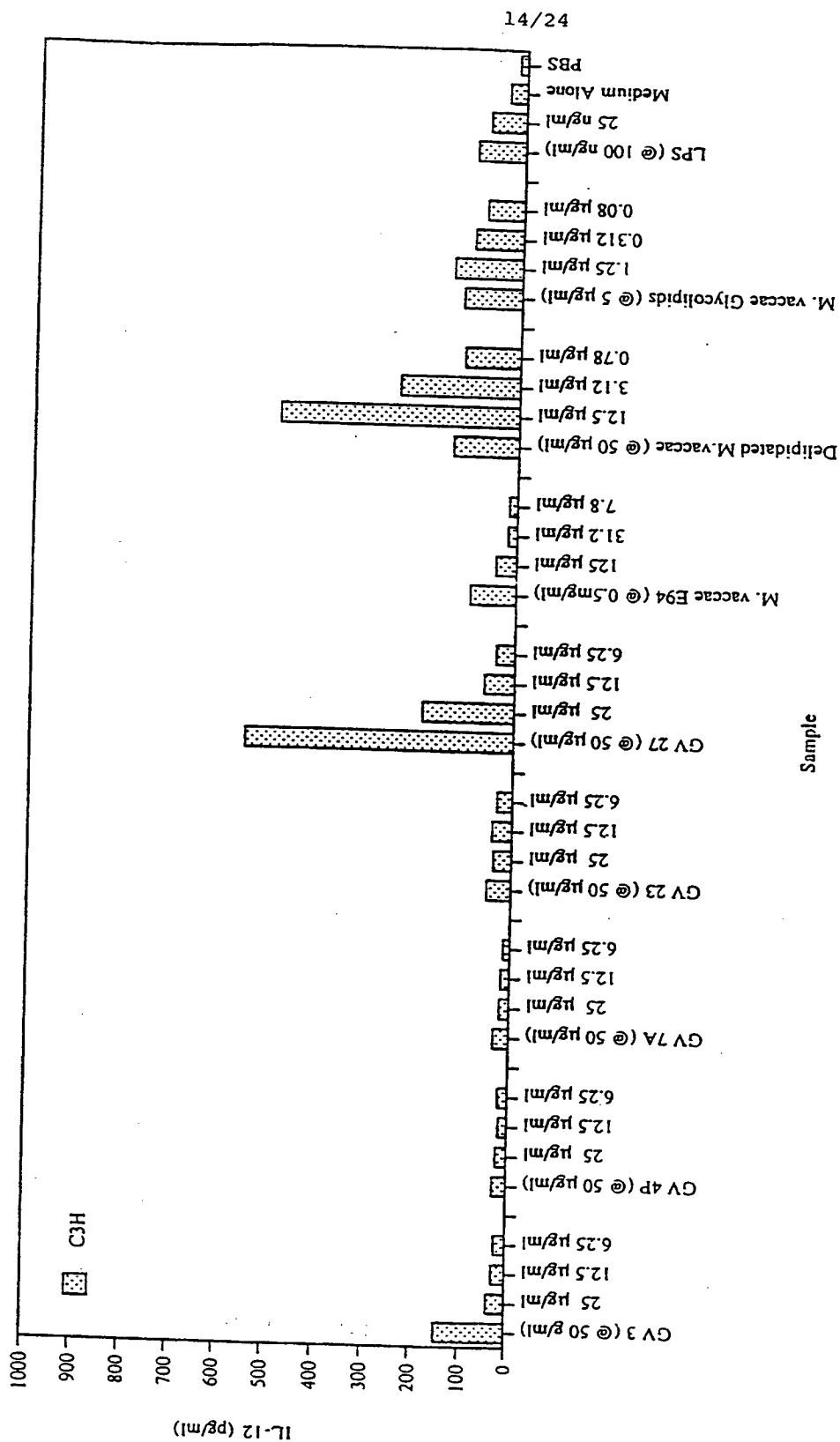
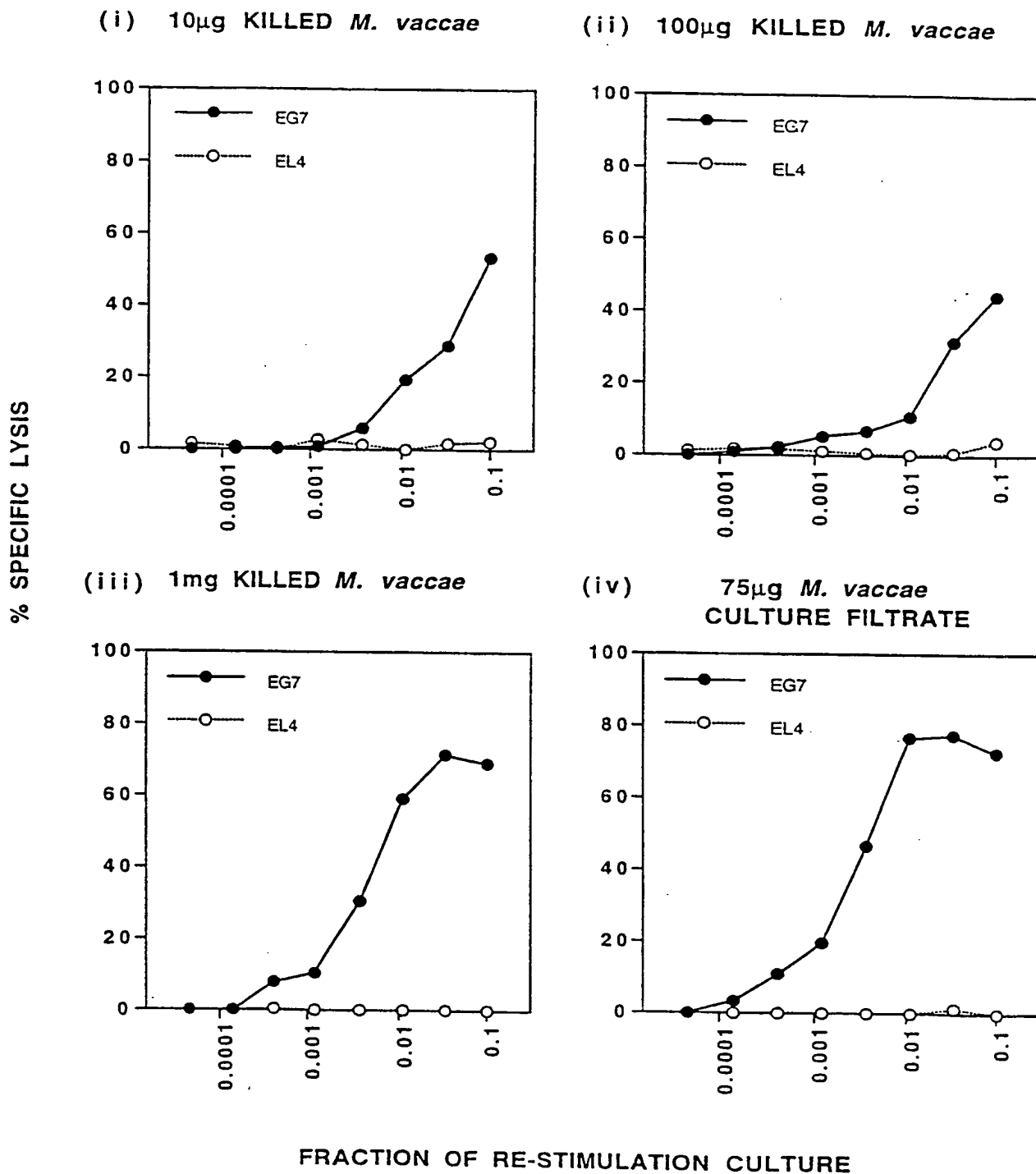


Figure 6C IL-12 dose-responses from C3H/HeJ peritoneal macrophages stimulated with purified GV proteins (97087-E04).





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Figure 7B

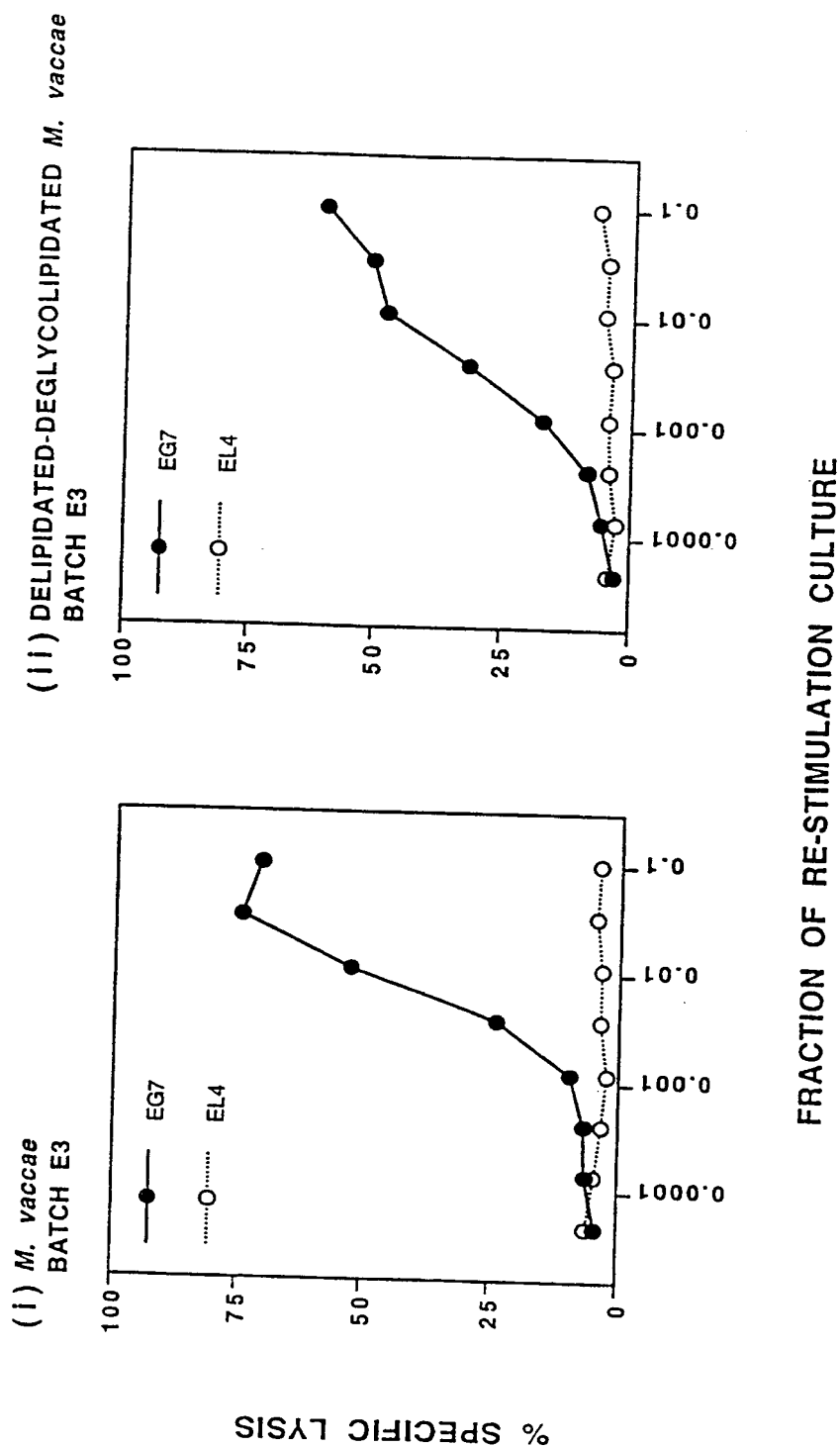
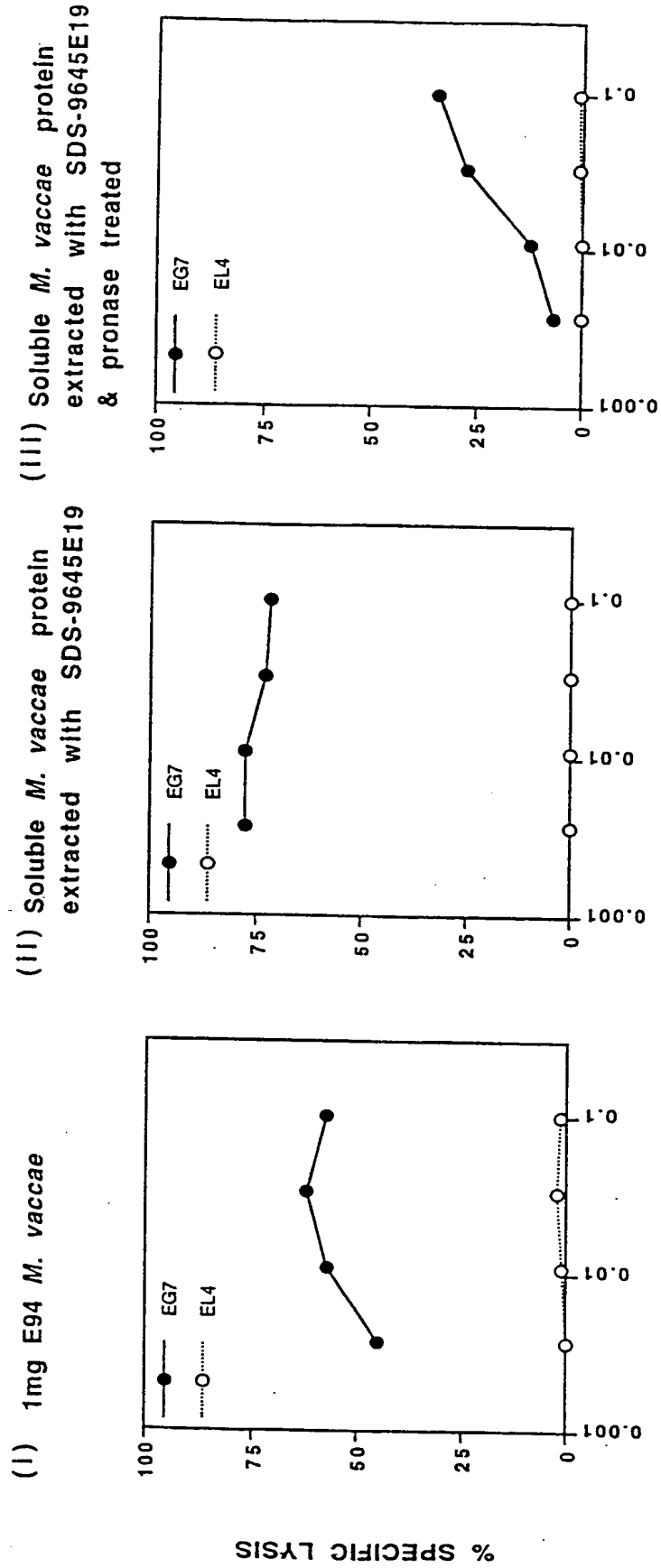
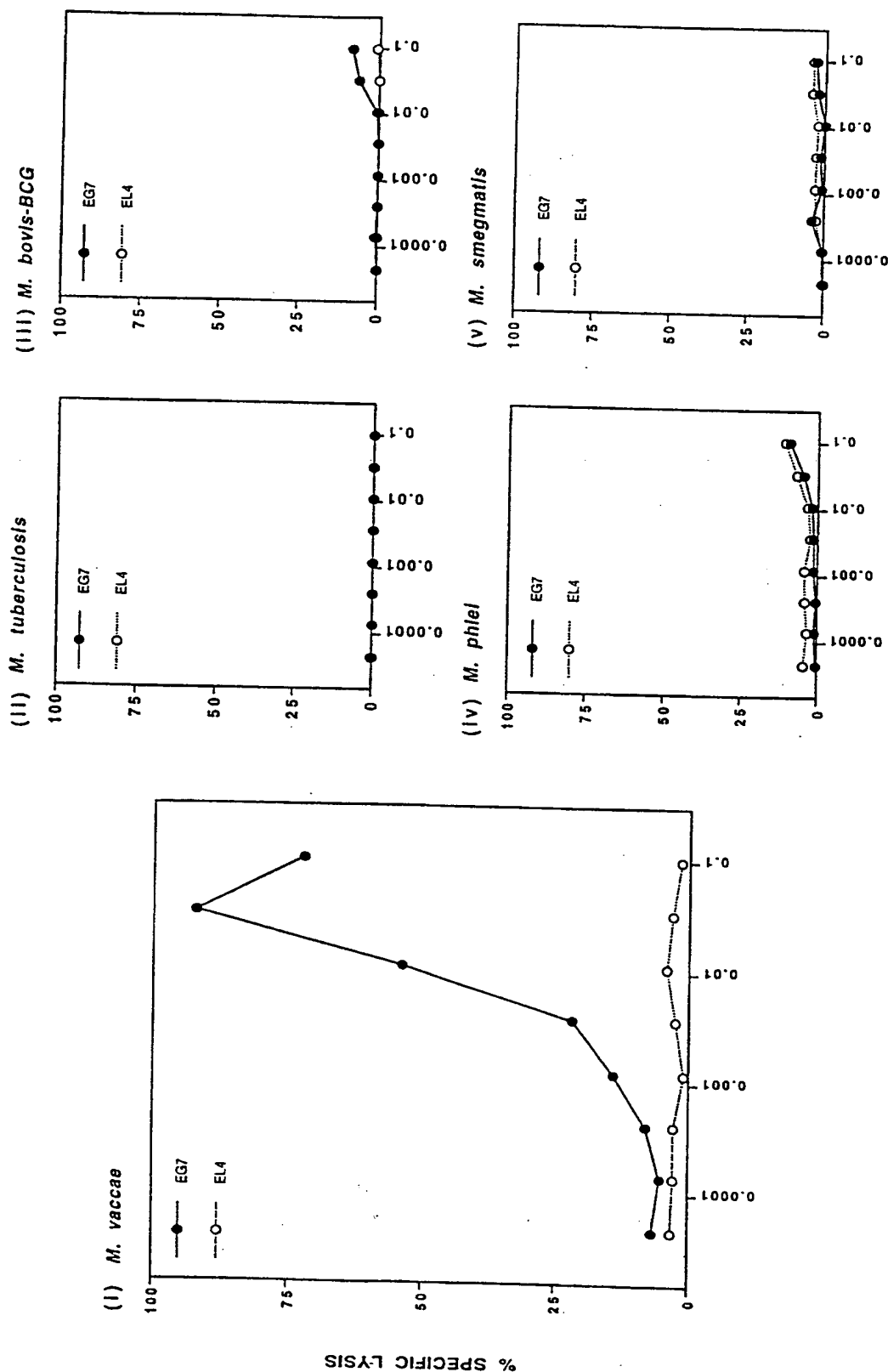


Figure 7C



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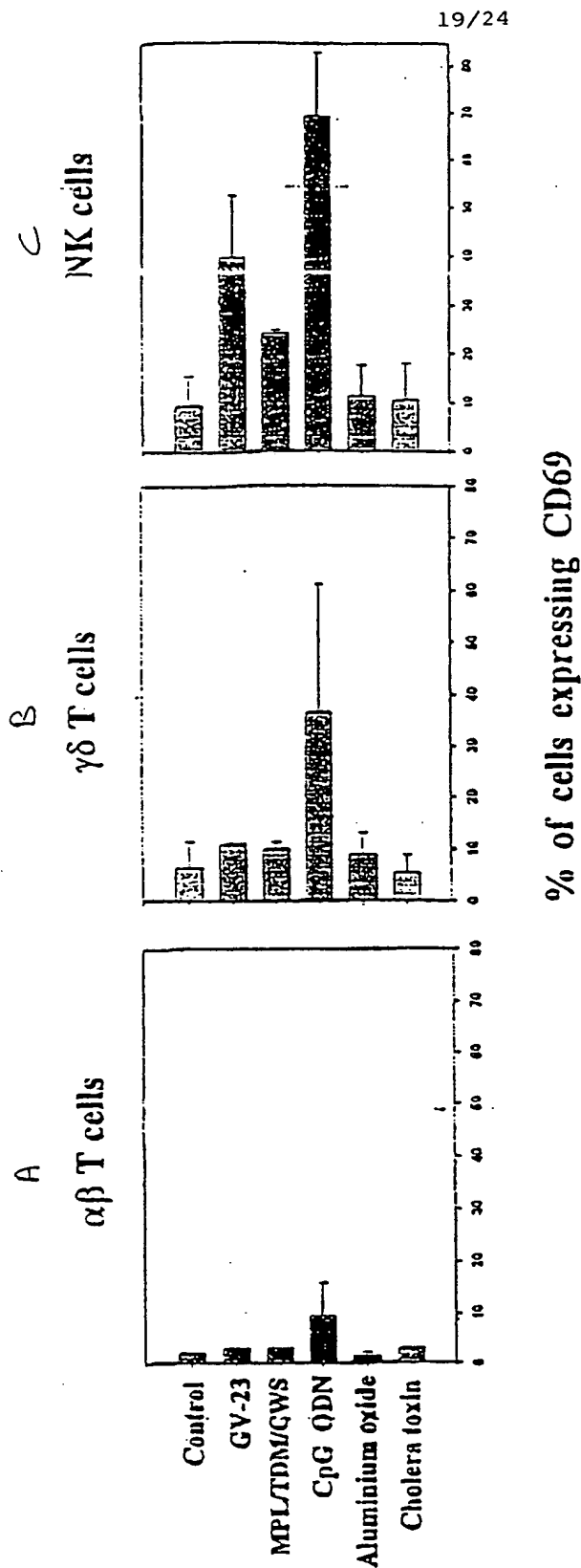
Figure 7D COMPARISON OF EFFECT OF HEAT KILLED *M. vaccae*, *M. tuberculosis*, *M. bovis*-BCG, *M. phlei* and *M. smegmatis* ON GENERATION OF OVA-SPECIFIC CTL



FRACTION OF RE-STIMULATION CULTURE

Fig. 8

The effect of GV-23 compared with 4 adjuvants on activation of lymphocyte subpopulations
(monitoring CD69 expression)



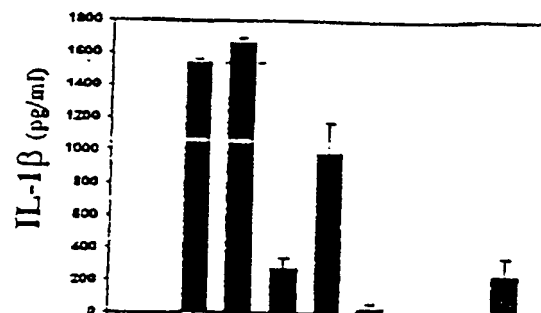
* Bar = Mean \pm S.E.
n = 2

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The effect of *M. vaccae* recombinant proteins on human cytokine production

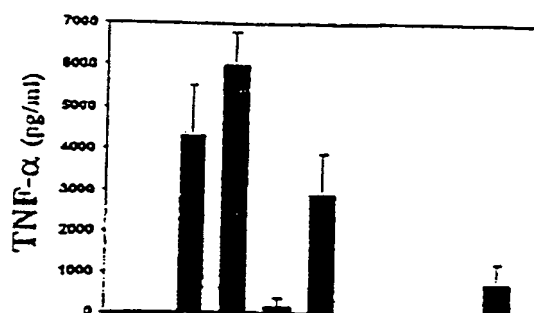
FIG. 9

A



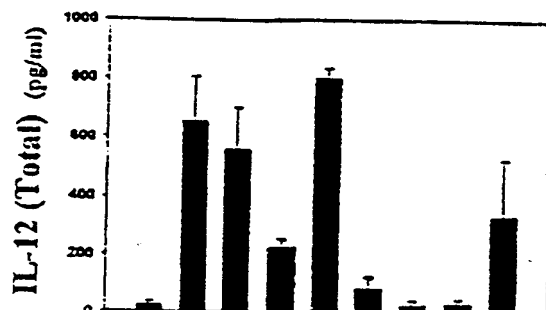
IL-1β

B



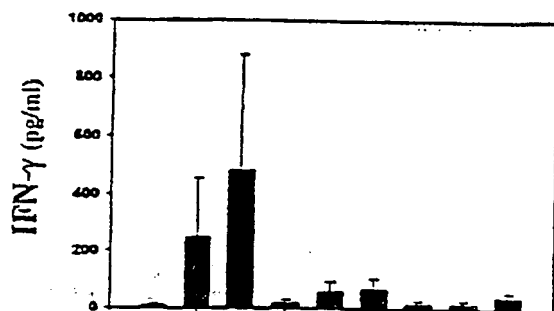
TNF-α

C



IL-12 (Total)

D



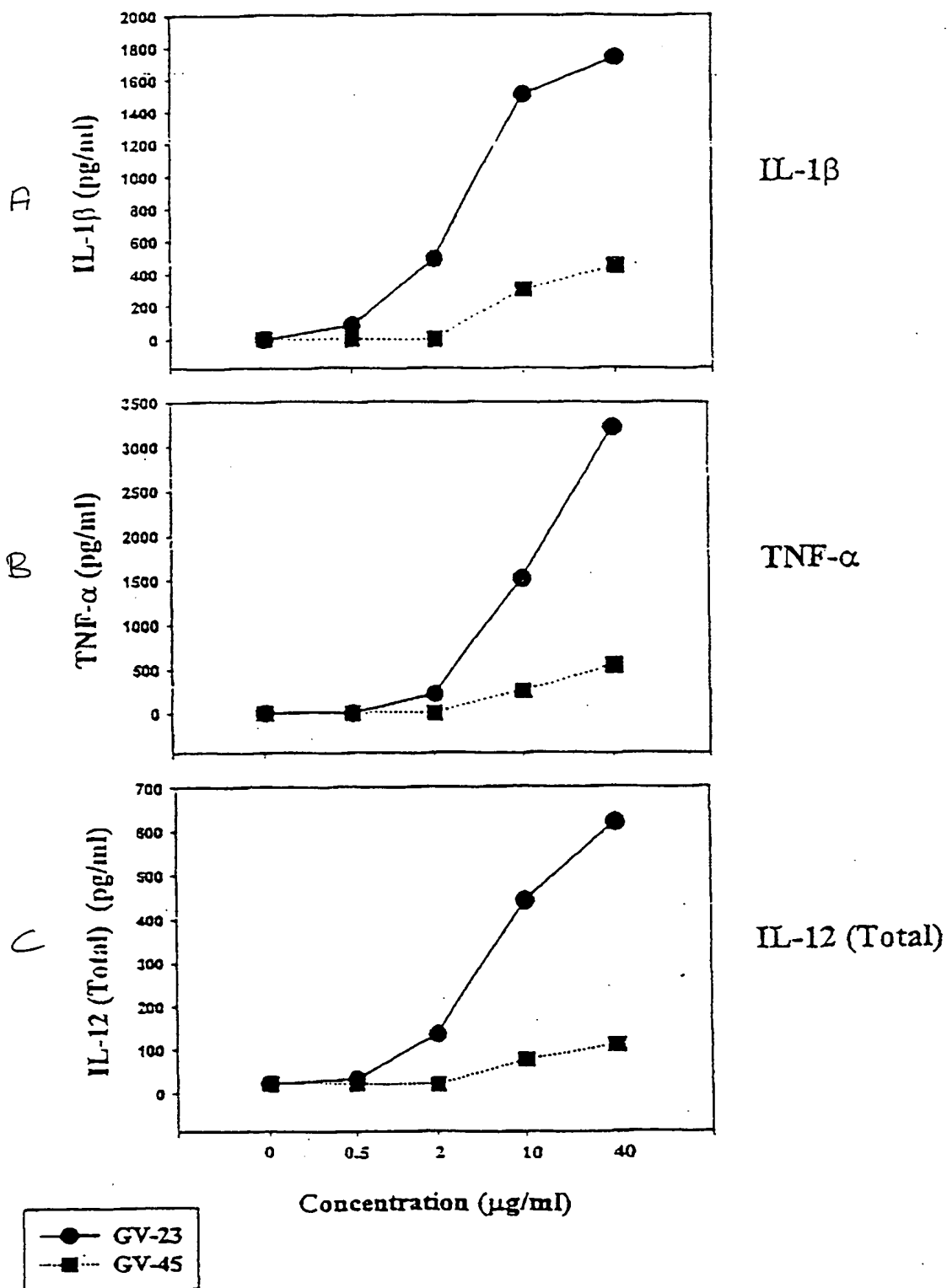
IFN-γ

* Bar = Mean +/- S.E.
n = 3

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Comparison of GV-23 and GV-45 on human cytokine induction

FIG. 10

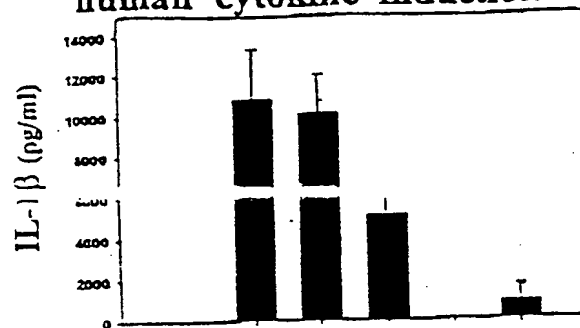


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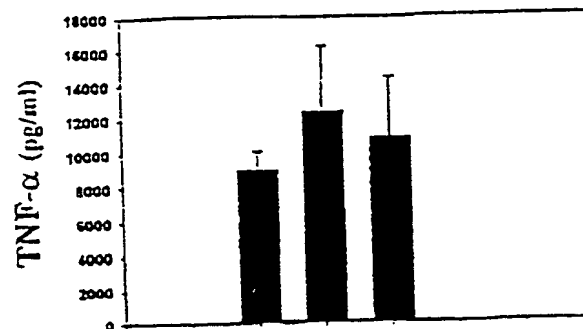
The effect of GV-23 compared with 4 adjuvants on
human cytokine induction

FIG 11

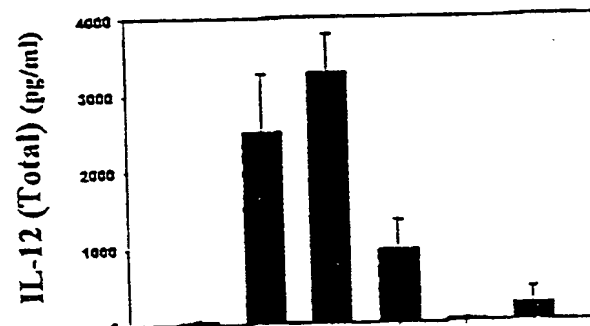
A

IL-1 β

B

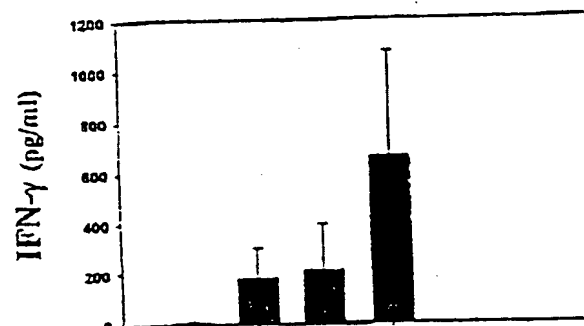
TNF- α

C



IL-12 (Total)

D

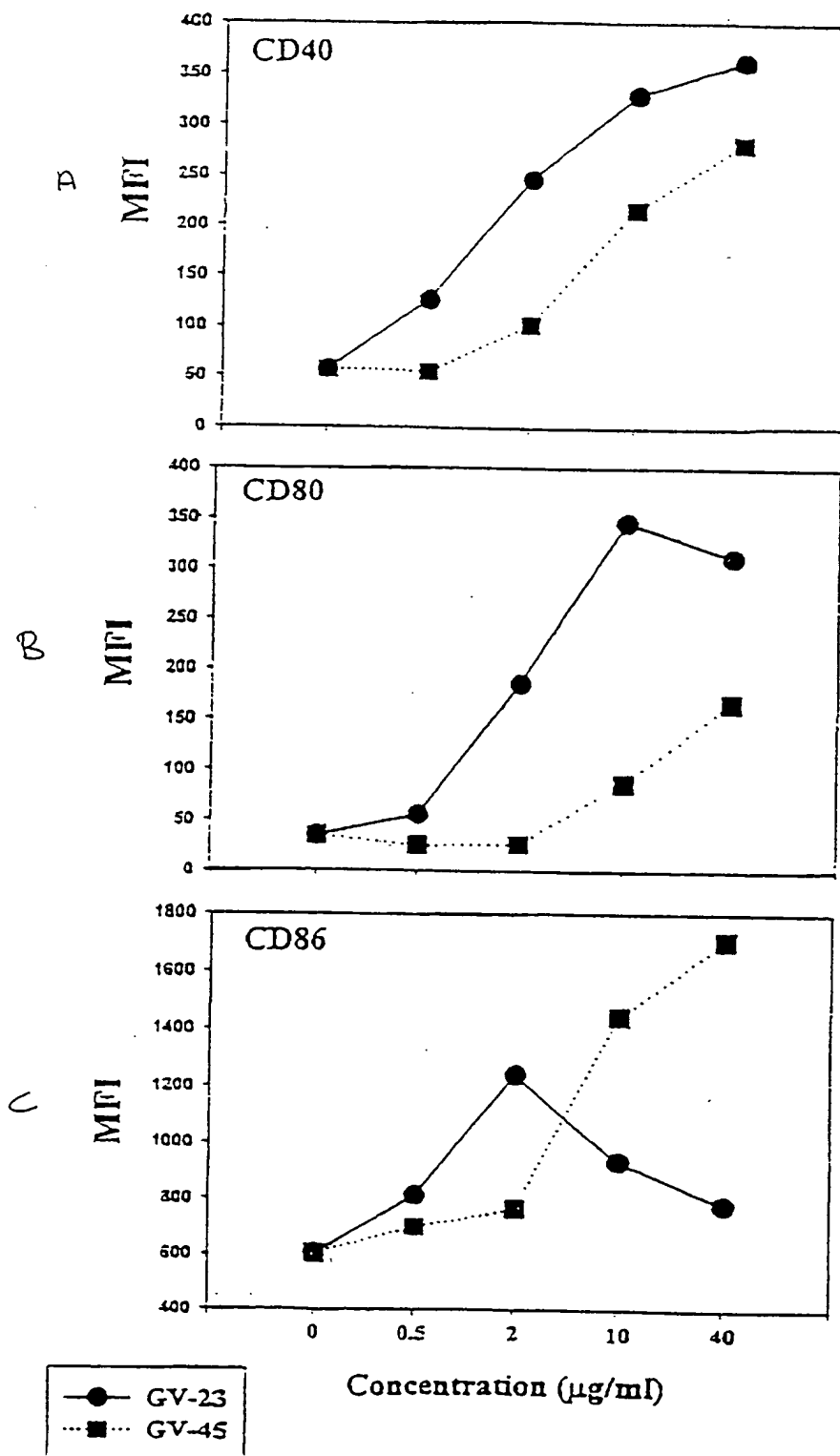
IFN- γ

* Bar = Mean \pm S.E.
n = 3

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Comparison of GV-23 and GV-45 on expression of co-stimulatory molecule expression on DC

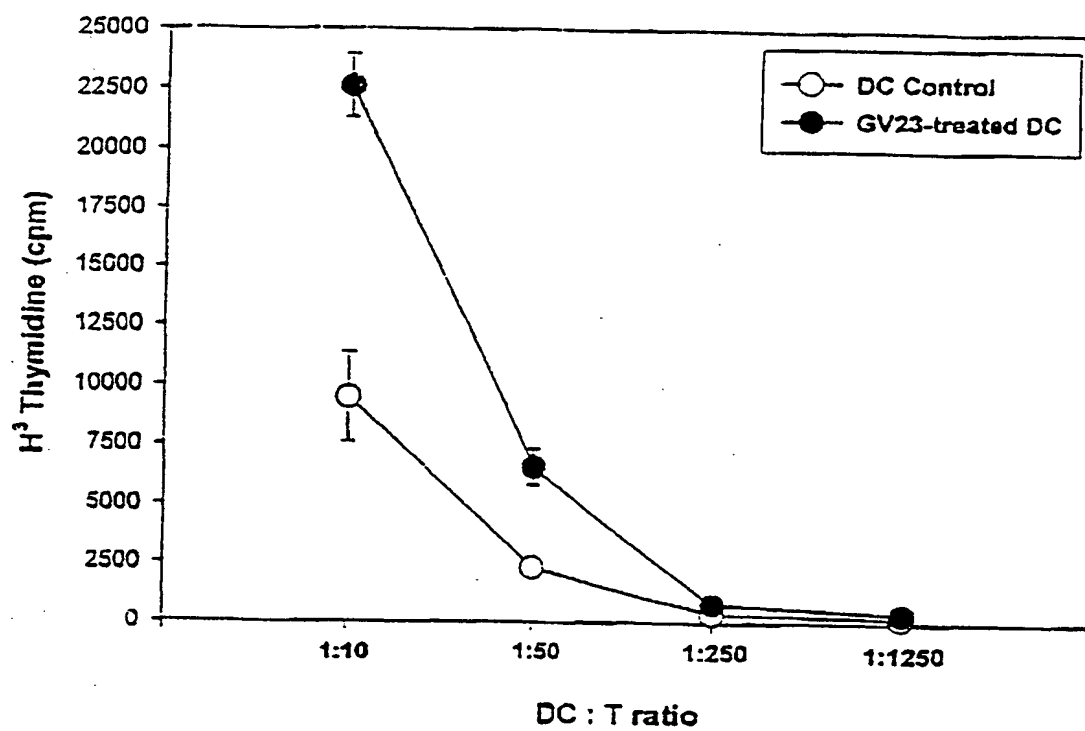
FIG. 12



SUBSTITUTE SHEET (Rule 26)

FIG. 13

MLR



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Watson, James D.
Visser, Elizabeth S.
Skinner, Margot A.
Prestidge, Ross L.

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Xaa	Arg	Ile	Asp												
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<213> Mycobacterium leprae

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Arg	Leu	Val	Val	Glu	Ala	Met	Gly	Val	Ala	Leu	Leu	Ser	Ala	Leu	Ile
		20					25					30			
Gly	Val	Val	Gly	Ser	Ala	Pro	Ala	Glu	Ala	Phe	Ser	Arg	Pro	Gly	Leu
	35					40				45					
Pro	Val	Glu	Tyr	Leu	Gln	Val	Pro	Ser	Pro	Ser	Met	Gly	Arg	Asp	Ile
	50				55					60					
Lys	Val	Gln	Phe	Gln	Asn	Gly	Gly	Ala	Asn	Ser	Pro	Ala	Leu	Tyr	Leu
65				70					75					80	
Leu	Asp	Gly	Leu	Arg	Ala	Gln	Asp	Asp	Phe	Ser	Gly	Trp	Asp	Ile	Asn
			85					90					95		
Thr	Thr	Ala	Phe	Glu	Trp	Tyr	Tyr	Gln	Ser	Gly	Ile	Ser	Val	Val	Met
		100						105					110		
Pro	Val	Gly	Gly	Gln	Ser	Ser	Phe	Tyr	Ser	Asp	Trp	Tyr	Ser	Pro	Ala
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Cys	Gly	Lys	Ala	Gly	Cys	Gln	Thr	Tyr	Lys	Trp	Glu	Thr	Phe	Leu	Thr
	130					135					140				
Ser	Glu	Leu	Pro	Glu	Tyr	Leu	Gln	Ser	Asn	Lys	Gln	Ile	Lys	Pro	Thr
145					150					155				160	
Gly	Ser	Ala	Ala	Val	Gly	Leu	Ser	Met	Ala	Gly	Leu	Ser	Ala	Leu	Thr
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	245	250
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	260	265
Gly Ser Asn Leu Lys Phe Gln Asp Ala Tyr Asn Gly Ala Gly Gly His		270
	275	280
Asn Ala Val Phe Asn Leu Asn Ala Asp Gly Thr His Ser Trp Glu Tyr		285
	290	295
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Met Ala Val Pro Arg Ser Gly		320
	325	

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Leu Pro Val Glu Tyr Leu Gln Val Pro Ser Pro Ser Met Gly Arg Asp
50 55 60
Ile Lys Val Gln Phe Gln Ser Gly Gly Ala Asn Ser Pro Ala Leu Tyr
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85 90 95
Asn Thr Pro Ala Phe Glu Trp Tyr Asp Gln Ser Gly Leu Ser Val Val
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Ala Cys Gly Lys Ala Gly Cys Gln Thr Tyr Lys Trp Glu Thr Phe Leu
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Thr Leu Ala Ile Tyr His Pro Gln Gln Phe Val Tyr Ala Gly Ala Met
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Ser Gly Leu Leu Asp Pro Ser Gln Ala Met Gly Pro Thr Leu Ile Gly
195 200 205
Leu Ala Met Gly Asp Ala Gly Gly Tyr Lys Ala Ser Asp Met Trp Gly
210 215 220
Pro Lys Glu Asp Pro Ala Trp Gln Arg Asn Asp Pro Leu Leu Asn Val
225 230 235 240
Gly Lys Leu Ile Ala Asn Asn Thr Arg Val Trp Val Tyr Cys Gly Asn

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			260					265					270				
Glu	Gly	Phe	Val	Arg	Thr	Ser	Asn	Ile	Lys	Phe	Gln	Asp	Ala	Tyr	Asn		
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Ala	Gly	Gly	Gly	His	Asn	Gly	Val	Phe	Asp	Phe	Pro	Asp	Ser	Gly	Thr		
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His	Ser	Trp	Glu	Tyr	Trp	Gly	Ala	Gln	Leu	Asn	Ala	Met	Lys	Pro	Asp		
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Gln	Phe	Gln	Ser	Gly	Gly	Asn	Asn	Ser	Pro	Ala	Val	Tyr	Leu	Leu	Asp		
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Gly	Leu	Arg	Ala	Gln	Asp	Asp	Tyr	Asn	Gly	Trp	Asp	Ile	Asn	Thr	Pro		
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Lys	Ala	Gly	Cys	Gln	Thr	Tyr	Lys	Trp	Glu	Thr	Phe	Leu	Thr	Ser	Glu		
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Ala	Tyr	His	Pro	Gln	Gln	Phe	Ile	Tyr	Ala	Gly	Ser	Leu	Ser	Ala	Leu		
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Leu	Asp	Pro	Ser	Gln	Gly	Met	Gly	Pro	Ser	Leu	Ile	Gly	Leu	Ala	Met		
	195					200						205					
Gly	Asp	Ala	Gly	Gly	Tyr	Lys	Ala	Ala	Asp	Met	Trp	Gly	Pro	Ser	Ser		
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Asp	Pro	Ala	Trp	Glu	Arg	Asn	Asp	Pro	Thr	Gln	Gln	Ile	Pro	Lys	Leu		
225					230					235					240		
Val	Ala	Asn	Asn	Thr	Arg	Leu	Trp	Val	Tyr	Cys	Gly	Asn	Gly	Thr	Pro		
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			260			265						270				
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Asn	Thr	Pro	Ala	Phe	Glu	Trp	Tyr	Asp	Gln	Ser	Gly	Leu	Ser	Val	Val 110
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Ala	Cys	Gly	Lys	Ala	Gly	Cys	Gln	Thr	Tyr	Lys	Trp	Glu	Thr	Phe	Leu 140
Thr 145	Ser	Glu	Leu	Pro	Gly	Trp	Leu	Gln	Ala	Asn	Arg	His	Val	Lys	Pro 160
Thr	Gly	Ser	Ala	Val	Val	Gly	Leu	Ser	Met	Ala	Ala	Ser	Ser	Ala	Leu 175
Thr	Leu	Ala	Ile	Tyr	His	Pro	Gln	Gln	Phe	Val	Tyr	Ala	Gly	Ala	Met 190
Ser	Gly	Leu	Leu	Asp	Pro	Ser	Gln	Ala	Met	Gly	Pro	Thr	Leu	Ile	Gly 205
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Glu	Gly	Phe	Val	Arg	Thr	Ser	Asn	Ile	Lys	Phe	Gln	Asp	Ala	Tyr	Asn 285
Ala	Gly	Gly	Gly	His	Asn	Gly	Val	Phe	Asp	Phe	Pro	Asp	Ser	Gly	Thr

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 Gly Gly Ala Ala Thr Ala Gly Ala Phe Ser Arg Pro Gly Leu Pro Val
 35 40 45
 Glu Tyr Leu Gln Val Pro Ser Pro Ser Met Gly Arg Asp Ile Lys Val
 50 55 60
 Gln Phe Gln Ser Gly Gly Asn Asn Ser Pro Ala Val Tyr Leu Leu Asp
 65 70 75 80
 Gly Leu Arg Ala Gln Asp Asp Tyr Asn Gly Trp Asp Ile Asn Thr Pro
 85 90 95
 Ala Phe Glu Trp Tyr Tyr Gln Ser Gly Leu Ser Ile Val Met Pro Val
 100 105 110
 Gly Gly Gln Ser Ser Phe Tyr Ser Asp Trp Tyr Ser Pro Ala Cys Gly
 115 120 125
 Lys Ala Gly Cys Gln Thr Tyr Lys Trp Glu Thr Leu Leu Thr Ser Glu
 130 135 140
 Leu Pro Gln Trp Leu Ser Ala Asn Arg Ala Val Lys Pro Thr Gly Ser
 145 150 155 160
 Ala Ala Ile Gly Leu Ser Met Ala Gly Ser Ser Ala Met Ile Leu Ala
 165 170 175
 Ala Tyr His Pro Gln Gln Phe Ile Tyr Ala Gly Ser Leu Ser Ala Leu
 180 185 190
 Leu Asp Pro Ser Gln Gly Met Gly Leu Ile Gly Leu Ala Met Gly Asp
 195 200 205
 Ala Gly Gly Tyr Lys Ala Ala Asp Met Trp Gly Pro Ser Ser Asp Pro
 210 215 220
 Ala Trp Glu Arg Asn Asp Pro Thr Gln Gln Ile Pro Lys Leu Val Ala
 225 230 235 240
 Asn Asn Thr Arg Leu Trp Val Tyr Cys Gly Asn Gly Thr Pro Asn Glu
 245 250 255
 Leu Gly Gly Ala Asn Ile Pro Ala Glu Phe Leu Glu Asn Phe Val Arg
 260 265 270
 Ser Ser Asn Leu Lys Phe Gln Asp Ala Tyr Lys Pro Ala Gly Gly His
 275 280 285
 Asn Ala Val Phe Asn Phe Pro Pro Asn Gly Thr His Ser Trp Glu Tyr
 290 295 300
 Trp Gly Ala Gln Leu Asn Ala Met Lys Gly Asp Leu Gln Ser Ser Leu

305

310

315

320

Gly Ala Gly

<210> 36

<211> 333

<212> PRT

<213> Mycobacterium leprae

<400> 36

```

Met Lys Phe Leu Gln Gln Met Arg Lys Leu Phe Gly Leu Ala Ala Lys
 1           5           10           15
Phe Pro Ala Arg Leu Thr Ile Ala Val Ile Gly Thr Ala Leu Leu Ala
      20           25           30
Gly Leu Val Gly Val Val Gly Asp Thr Ala Ile Ala Val Ala Phe Ser
      35           40           45
Lys Pro Gly Leu Pro Val Glu Tyr Leu Gln Val Pro Ser Pro Ser Met
      50           55           60
Gly His Asp Ile Lys Ile Gln Phe Gln Gly Gly Gly Gln His Ala Val
65           70           75           80
Tyr Leu Leu Asp Gly Leu Arg Ala Gln Glu Asp Tyr Asn Gly Trp Asp
      85           90           95
Ile Asn Thr Pro Ala Phe Glu Glu Tyr Tyr His Ser Gly Leu Ser Val
      100          105          110
Ile Met Pro Val Gly Gly Gln Ser Ser Phe Tyr Ser Asn Trp Tyr Gln
      115          120          125
Pro Ser Gln Gly Asn Gly Gln His Tyr Thr Tyr Lys Trp Glu Thr Phe
      130          135          140
Leu Thr Gln Glu Met Pro Ser Trp Leu Gln Ala Asn Lys Asn Val Leu
145          150          155          160
Pro Thr Gly Asn Ala Ala Val Gly Leu Ser Met Ser Gly Ser Ser Ala
      165          170          175
Leu Ile Leu Ala Ser Tyr Tyr Pro Gln Gln Phe Pro Tyr Ala Ala Ser
      180          185          190
Leu Ser Gly Phe Leu Asn Pro Ser Glu Gly Trp Trp Pro Thr Met Ile
      195          200          205
Gly Leu Ala Met Asn Asp Ser Gly Gly Tyr Asn Ala Asn Ser Met Trp
      210          215          220
Gly Pro Ser Thr Asp Pro Ala Trp Lys Arg Asn Asp Pro Met Val Gln
225          230          235          240
Ile Pro Arg Leu Val Ala Asn Asn Thr Arg Ile Trp Val Tyr Cys Gly
      245          250          255
Asn Gly Ala Pro Asn Glu Leu Gly Gly Asp Asn Ile Pro Ala Lys Phe
      260          265          270
Leu Glu Ser Leu Thr Leu Ser Thr Asn Glu Ile Phe Gln Asn Thr Tyr
      275          280          285
Ala Ala Ser Gly Gly Arg Asn Gly Val Phe Asn Phe Pro Pro Asn Gly
      290          295          300
Thr His Ser Trp Pro Tyr Trp Asn Gln Gln Leu Val Ala Met Lys Pro
305          310          315          320
Asp Ile Gln Gln Ile Leu Asn Gly Ser Asn Asn Asn Ala
      325          330

```

<210> 37
 <211> 340
 <212> PRT
 <213> Mycobacterium tuberculosis

<400> 37
 Met Thr Phe Phe Glu Gln Val Arg Arg Leu Arg Ser Ala Ala Thr Thr
 1 5 10 15
 Leu Pro Arg Arg Val Ala Ile Ala Ala Met Gly Ala Val Leu Val Tyr
 20 25 30
 Gly Leu Val Gly Thr Phe Gly Gly Pro Ala Thr Ala Gly Ala Phe Ser
 35 40 45
 Arg Pro Gly Leu Pro Val Glu Tyr Leu Gln Val Pro Ser Ala Ser Met
 50 55 60
 Gly Arg Asp Ile Lys Val Gln Phe Gln Gly Gly Gly Pro His Ala Val
 65 70 75 80
 Tyr Leu Leu Asp Gly Leu Arg Ala Gln Asp Asp Tyr Asn Gly Trp Asp
 85 90 95
 Ile Asn Thr Pro Ala Phe Glu Glu Tyr Tyr Gln Ser Gly Leu Ser Val
 100 105 110
 Ile Met Pro Val Gly Gly Gln Ser Ser Phe Tyr Thr Asp Trp Tyr Gln
 115 120 125
 Pro Ser Gln Ser Asn Gly Gln Asn Tyr Thr Tyr Lys Trp Glu Thr Phe
 130 135 140
 Leu Thr Arg Glu Met Pro Ala Trp Leu Gln Ala Asn Lys Gly Val Ser
 145 150 155 160
 Pro Thr Gly Asn Ala Val Gly Leu Ser Met Ser Gly Gly Ser Ala
 165 170 175
 Leu Ile Leu Ala Ala Tyr Tyr Pro Gln Gln Phe Pro Tyr Ala Ala Ser
 180 185 190
 Leu Ser Gly Phe Leu Asn Pro Ser Glu Gly Trp Trp Pro Thr Leu Ile
 195 200 205
 Gly Leu Ala Met Asn Asp Ser Gly Gly Tyr Asn Ala Asn Ser Met Trp
 210 215 220
 Gly Pro Ser Ser Asp Pro Ala Trp Lys Arg Asn Asp Pro Met Val Gln
 225 230 235 240
 Ile Pro Arg Leu Val Ala Asn Asn Thr Arg Ile Trp Val Tyr Cys Gly
 245 250 255
 Asn Gly Thr Pro Ser Asp Leu Gly Gly Asp Asn Ile Pro Ala Lys Phe
 260 265 270
 Leu Glu Gly Leu Thr Leu Arg Thr Asn Gln Thr Phe Arg Asp Thr Tyr
 275 280 285
 Ala Ala Asp Gly Gly Arg Asn Gly Val Phe Asn Phe Pro Pro Asn Gly
 290 295 300
 Thr His Ser Trp Pro Tyr Trp Asn Glu Gln Leu Val Ala Met Lys Ala
 305 310 315 320
 Asp Ile Gln His Val Leu Asn Gly Ala Thr Pro Pro Ala Ala Pro Ala
 325 330 335
 Ala Pro Ala Ala
 340

<210> 38
 <211> 20

<212> DNA
<213> Artificial Sequence

<220>
<223> Probe made in a lab

<400> 38
agcggctggg acatcaacac

20

<210> 39
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe made in a lab

<400> 39
cagacgcggg tgttggtggc

20

<210> 40
<211> 1211
<212> DNA
<213> Mycobacterium vaccae

<400> 40
ggtaccggaa gctggaggat tgacgggatg agacttcttg acaggattcg tgggccttgg 60
gcacgccgtt tcggcgctcg ggctgtcgcg acagcgatga tgcttgcctt ggtgggcctg 120
gctggagggg cggcgaccgc cggagcattc tcccggccag gtctgccggg ggagtacctg 180
atggtgcctt cgccgtcgat ggggcgcgac atcaagatcc agttccagag cggtggcgag 240
aactcgccgg ctctctacct gctcgacggc ctgcgtgcgc aggaggactt caacggctgg 300
gacatcaaca ctcaggcttt cgagtgggtc ctgcacagcg gcatctccgt ggtgatgccg 360
gtcggtgggc agtccagctt ctacaccgac tgggtacgcc cgcgccgtaa caagggcccg 420
accgtgacct acaagtggga gaccttcctg acccaggagc tcccgggctg gctgcaggcc 480
aaccgcgcgg tcaagccgac cggcagcggc cctgtcggtc tgtcgatggc ggggttcggc 540
gcgctgaacc tggcgacctg gcaccgggag cagttcatct acgcgggctc gatgtccggc 600
ttcctgaacc cctccgaggg ctggtggccg ttcctgatca acatctcgat ggggtgacgc 660
ggcggcttca aggcgcagca catgtggggc aagaccgagg ggatcccaac agcgggttga 720
cagcgcaacg atccgatgct gaacatccc accctggctg ccaacaacac ccgtatctgg 780
gtctactgcg gtaacggcca gccaccgag ctccggcggc gcgacctgcc cgccacgttc 840
ctcgaaggtc tgaccatccg caccaacgag accttcgcgc acaactacat cgccgcgggt 900
ggccacaacg gtgtgttcaa cttcccggcc aacggcacgc acaactgggc gtactggggg 960
cgcgagctgc aggcgatgaa gcctgacctg caggcgcacc ttctctgacg gttgcacgaa 1020
acgaagcccc cggccgattg cggccgaggg ttctgctgct cggggctact gtggccgaca 1080
taaccgaaat caacgcgatg gtggctcatc aggaacgcc aggggggtcat tgcgctacga 1140
cacgaggtgg gcgagcaatc cttcctgccc gacggagagg tcaacatcca cgtcgagtac 1200
tccagcgtga a 1211

<210> 41
<211> 485
<212> DNA
<213> Mycobacterium vaccae

<400> 41

```

agcggctggg acatcaacac cgccgccttc gagtggtagc tcgactcggg tctcgcgggtg      60
atcatgcccg tcggcgggca gtccagcttc tacagcgact ggtacagccc ggcctgcgggt      120
aaggccgggt gccagacctt caagtgggag acgttcctga cccaggagct gccggcctac      180
ctcgcggcca acaagggggt cgacccgaac cgcaacgcgg ccgtcgggtc gtccatggcc      240
ggttcggcgg cgctgacgct ggcgatctac caccgcagc agttccagta cgcggggtcg      300
ctgtcgggct acctgaaccc gtccgagggg tgggtggcca tgctgatcaa catctcgatg      360
ggtgacgcgg gcggctacaa ggccaacgac atgtggggtc caccgaagga cccgagcagc      420
gcctggaagc gcaacgaccc gatggtcaac atcggcaagc tgggtggcaa caacaccccc      480
ctctc                                         485

```

<210> 42

<211> 1052

<212> DNA

<213> Mycobacterium vaccae

<400> 42

```

gttgatgaga aagggtgggtt gtttgccgtt atgaagttca cagagaagtg gcgggggtcc      60
gcaaaggcgg cgatgcaccg ggtgggcgtt gccgatatgg ccgccgttgc gctgcccggga      120
ctgatcggct tcgcccggggg ttccggcaacg gccggggcat tctcccggcc cgggtcttct      180
gtcgagtacc tcgacgtggt ctcccgctcg atgggccgag acatccgggt ccagttccag      240
ggtggcggta ctcatgcggg ctacctgctc gacggtctgc gtgccaggga cgactacaac      300
ggctgggaca tcaacacccc tgcgttcgag tggttctacg agtccggctt gtcgacgatc      360
atgccggtcg gcggacagtc cagcttctac agcgactggt accagccgtc tcgggggcaac      420
gggcagaact acacctacaa gtgggagacg ttcttgaccc aggagctgcc gacgtggctg      480
gaggccaacc gcggagtgtc gcgcaccggc aacgcgttcg tcggcctgtc gatggcgggc      540
agcgcggcgc tgacctacgc gatccatcac ccgcagcagt tcatctacgc ctcgctcgctg      600
tcaggcttcc tgaacccgtc cgagggctgg tggccgatgc tgatcgggct ggcatgaac      660
gacgcaggcg gcttcaacgc cgagagcatg tggggcccgt cctcggaccc ggctggaag      720
cgcaacgacc cgatggtcaa catcaaccag ctggtggcca acaacacccg gatctggatc      780
tactgcggca ccggcacccc gtccgagctg gacaccggga ccccgggcca gaacctgatg      840
gccgcgcagt tcctcgaagg attcacgttg cggaccaaca tcgccttccg tgacaactac      900
atcgcagccg gcggcaccaa cgggtgtctc aacttcccgg cctcgggcac ccacagctgg      960
gggtactggg ggcagcagct gcagcagatg aagcccgcga tccagcgggt tctgggagct      1020
caggccaccg cctagccacc caccacacac cc                                         1052

```

<210> 43

<211> 326

<212> PRT

<213> Mycobacterium vaccae

<400> 43

```

Met Arg Leu Leu Asp Arg Ile Arg Gly Pro Trp Ala Arg Arg Phe Gly
 1           5           10          15
Val Val Ala Val Ala Thr Ala Met Met Pro Ala Leu Val Gly Leu Ala
          20          25          30
Gly Gly Ser Ala Thr Ala Gly Ala Phe Ser Arg Pro Gly Leu Pro Val
          35          40          45
Glu Tyr Leu Met Val Pro Ser Pro Ser Met Gly Arg Asp Ile Lys Ile
          50          55          60
Gln Phe Gln Ser Gly Gly Glu Asn Ser Pro Ala Leu Tyr Leu Leu Asp
65          70          75          80
Gly Leu Arg Ala Gln Glu Asp Phe Asn Gly Trp Asp Ile Asn Thr Gln

```

```
<210> 44
<211> 161
<212> PRT
<213> Mycobacterium vaccae
```

<400> 44															
Ser 1	Gly	Trp	Asp	Ile 5	Asn	Thr	Ala	Ala	Phe 10	Glu	Trp	Tyr	Val	Asp 15	Ser
Gly	Leu	Ala	Val	Ile	Met	Pro	Val	Gly 25	Gly	Gln	Ser	Ser	Phe 30	Tyr	Ser
Asp	Trp	Tyr	Ser	Pro	Ala	Cys	Gly 40	Lys	Ala	Gly	Cys	Gln 45	Thr	Tyr	Lys
Trp	Glu	Thr	Phe	Leu	Thr	Gln	Glu	Leu	Pro	Ala	Tyr 60	Leu	Ala	Ala	Asn
Lys 65	Gly	Val	Asp	Pro	Asn 70	Arg	Asn	Ala	Ala	Val 75	Gly	Leu	Ser	Met	Ala 80
Gly	Ser	Ala	Ala	Leu 85	Thr	Leu	Ala	Ile	Tyr 90	His	Pro	Gln	Gln	Phe 95	Gln
Tyr	Ala	Gly	Ser	Leu	Ser	Gly	Tyr	Leu 105	Asn	Pro	Ser	Glu	Gly 110	Trp	Trp
Pro	Met	Leu	Ile	Asn	Ile	Ser	Met	Gly	Asp	Ala	Gly	Gly	Tyr	Lys	Ala

115	120	125
Asn Asp Met Trp Gly Pro Pro Lys Asp Pro Ser Ser Ala Trp Lys Arg		
130	135	140
Asn Asp Pro Met Val Asn Ile Gly Lys Leu Val Ala Asn Asn Thr Pro		
145	150	155
Leu		160

<210> 45
 <211> 334
 <212> PRT
 <213> Mycobacterium vaccae

<400> 45

Met Lys Phe Thr Glu Lys Trp Arg Gly Ser Ala Lys Ala Ala Met His		
1	5	10
Arg Val Gly Val Ala Asp Met Ala Ala Val Ala Leu Pro Gly Leu Ile		15
	20	25
Gly Phe Ala Gly Gly Ser Ala Thr Ala Gly Ala Phe Ser Arg Pro Gly		30
	35	40
Leu Pro Val Glu Tyr Leu Asp Val Phe Ser Pro Ser Met Gly Arg Asp		45
	50	55
Ile Arg Val Gln Phe Gln Gly Gly Gly Thr His Ala Val Tyr Leu Leu		60
65	70	75
Asp Gly Leu Arg Ala Gln Asp Asp Tyr Asn Gly Trp Asp Ile Asn Thr		80
	85	90
Pro Ala Phe Glu Trp Phe Tyr Glu Ser Gly Leu Ser Thr Ile Met Pro		95
	100	105
Val Gly Gly Gln Ser Ser Phe Tyr Ser Asp Trp Tyr Gln Pro Ser Arg		110
	115	120
Gly Asn Gly Gln Asn Tyr Thr Tyr Lys Trp Glu Thr Phe Leu Thr Gln		125
	130	135
Glu Leu Pro Thr Trp Leu Glu Ala Asn Arg Gly Val Ser Arg Thr Gly		140
145	150	155
Asn Ala Phe Val Gly Leu Ser Met Ala Gly Ser Ala Ala Leu Thr Tyr		160
	165	170
Ala Ile His His Pro Gln Gln Phe Ile Tyr Ala Ser Ser Leu Ser Gly		175
	180	185
Phe Leu Asn Pro Ser Glu Gly Trp Trp Pro Met Leu Ile Gly Leu Ala		190
	195	200
Met Asn Asp Ala Gly Gly Phe Asn Ala Glu Ser Met Trp Gly Pro Ser		205
210	215	220
Ser Asp Pro Ala Trp Lys Arg Asn Asp Pro Met Val Asn Ile Asn Gln		225
225	230	235
Leu Val Ala Asn Asn Thr Arg Ile Trp Ile Tyr Cys Gly Thr Gly Thr		240
	245	250
Pro Ser Glu Leu Asp Thr Gly Thr Pro Gly Gln Asn Leu Met Ala Ala		255
	260	265
Gln Phe Leu Glu Gly Phe Thr Leu Arg Thr Asn Ile Ala Phe Arg Asp		270
	275	280
Asn Tyr Ile Ala Ala Gly Gly Thr Asn Gly Val Phe Asn Phe Pro Ala		285
290	295	300
Ser Gly Thr His Ser Trp Gly Tyr Trp Gly Gln Gln Leu Gln Gln Met		


```
<210> 46
<211> 795
<212> DNA
<213> Mycobacterium vaccae
```

```
<210> 47
<211> 142
<212> PRT
<213> Mycobacterium vaccae
```

<210>	48
<211>	300
<212>	DNA

<213> Mycobacterium vaccae

<400> 48

gccagtgcgc	caacgggtttt	catcgatgcc	gcacacaacc	ccggtggggc	ctgcgcttgc	60
cgaaggctgc	gcgacgagtt	cgacttccgg	tatctcgtcg	gcgtcgtctc	ggtgatgggg	120
gacaaggacg	tggacgggat	ccgccaggac	ccgggcgtgc	cggacgggcg	cggctcgcga	180
ctgttcgtct	cgggcgacaa	ccttcgaaa	ggtgcggcgc	tcaacacgat	ccagatcgcc	240
gagctgctgg	ccgccagtt	gtaagtgttc	cgccgaaatt	gcattccacg	ccgataatcg	300

<210> 49

<211> 563

<212> DNA

<213> Mycobacterium vaccae

<400> 49

ggatcctcgg	ccggctcaag	agtccgcgcc	gaggtggatg	tgacgctgga	cggctacgag	60
ttcagtcggg	cctgcgaggc	gctgtaccac	ttcgccctgg	acgagttctg	cgactggtat	120
gtcgaagctt	ccaaagtgc	actgggtgaa	ggtttctcgc	acaccacggc	cgtgttggcc	180
accgtgctcg	atgtgctgct	caagcttctg	cacccggtca	tgccgttctg	caccgaggtg	240
ctgtggaagg	ccctgaccgg	gcgggccggc	gcgagcgaac	gtctgggaaa	tgtggagtca	300
ctggctcgtc	cggactggcc	cacgcccacc	ggatacgccg	tggatcaggc	tgccgcacaa	360
cggatcgccg	acaccagaa	gttgatcacc	gaggtgcgcc	ggttcgcgag	cgatcagggg	420
ctggccgacc	gccagcgggt	gcctgcccgg	ttgtccggca	tcgacaccgc	gggtctggac	480
gcccattgtc	cggcggtgcg	cgcgctggcc	tggtctgacc	gaggggtgat	agggcttcac	540
cgctccgaa	tcggctcgagg	tgc				563

<210> 50

<211> 434

<212> DNA

<213> Mycobacterium vaccae

<400> 50

gggcggggcc	cgaggatgag	caagttcgaa	gtcgtcaccg	ggatggcggt	cgcggctttc	60
gccgacgcgc	ccatcgacgt	cgccgtcgtc	gaggtcgggc	tcgggtggcg	ctgggacgcg	120
acgaacgtgg	tgaacgcacc	ggtcgcggtc	atcaccccca	tcgggggtgga	ccacaccgac	180
tacctcggtg	acacgatcgc	cgagatcgcc	ggggagaagg	ccggaaatca	tcacccgcca	240
gccgacgacc	tggtgccgac	cgacaccgtc	gccgtgctgg	cgcggcaggt	tcccagggcc	300
atggaggtgc	tgtgtggcca	ggcgggtgcg	tcggatgcgg	ctgtagcgcg	cgaggattcg	360
gagtgcgcgg	tgtgtggccg	tcaggtcgcc	atcggcggca	gctgctccgg	ttgcaggggc	420
tcgggtggcg	ctac					434

<210> 51

<211> 438

<212> DNA

<213> Mycobacterium vaccae

<400> 51

ggatcccact	cccgcgcggg	cggcgggccg	ctggtacggc	cattccagcg	tgctgatcga	60
ggtcgacggc	taccgctgct	tggccgaccc	ggtgtggagc	aacagatggt	cgccctcacg	120
ggcggtcgga	ccgcagcgca	tgcacgacgt	cccgggtgcc	ctggaggcgc	ttcccgcgct	180
ggacgcgggt	gtgatcgcca	acgaccacta	cgaccacctc	gacatcgaca	ccatcgctcg	240
gttggcgcac	acccagcggg	ccccgttcgt	ggtgccggtt	ggcatcgggc	cacacctgcg	300
caagtggggc	gtccccgagg	cgcggatcgt	cgagttggac	tggcacgaag	cccaccgcat	360

cgacgacctg acgctggtct gcacccccgc ccggcacttc tccggccggt tgttctcccg 420
cgactcgacg ctgtgggc 438

<210> 52
<211> 87
<212> PRT
<213> Mycobacterium vaccae

<400> 52
Ala Ser Ala Pro Thr Val Phe Ile Asp Ala Ala His Asn Pro Gly Gly
1 5 10 15
Pro Cys Ala Cys Arg Arg Leu Arg Asp Glu Phe Asp Phe Arg Tyr Leu
20 25 30
Val Gly Val Val Ser Val Met Gly Asp Lys Asp Val Asp Gly Ile Arg
35 40 45
Gln Asp Pro Gly Val Pro Asp Gly Arg Gly Leu Ala Leu Phe Val Ser
50 55 60
Gly Asp Asn Leu Arg Lys Gly Ala Ala Leu Asn Thr Ile Gln Ile Ala
65 70 75 80
Glu Leu Leu Ala Ala Gln Leu
85

<210> 53
<211> 175
<212> PRT
<213> Mycobacterium vaccae

<400> 53
Gly Ser Ser Ala Gly Ser Arg Val Arg Ala Glu Val Asp Val Thr Leu
1 5 10 15
Asp Gly Tyr Glu Phe Ser Arg Ala Cys Glu Ala Leu Tyr His Phe Ala
20 25 30
Trp Asp Glu Phe Cys Asp Trp Tyr Val Glu Leu Ala Lys Val Gln Leu
35 40 45
Gly Glu Gly Phe Ser His Thr Thr Ala Val Leu Ala Thr Val Leu Asp
50 55 60
Val Leu Leu Lys Leu Leu His Pro Val Met Pro Phe Val Thr Glu Val
65 70 75 80
Leu Trp Lys Ala Leu Thr Gly Arg Ala Gly Ala Ser Glu Arg Leu Gly
85 90 95
Asn Val Glu Ser Leu Val Val Ala Asp Trp Pro Thr Pro Thr Gly Tyr
100 105 110
Ala Leu Asp Gln Ala Ala Ala Gln Arg Ile Ala Asp Thr Gln Lys Leu
115 120 125
Ile Thr Glu Val Arg Arg Phe Arg Ser Asp Gln Gly Leu Ala Asp Arg
130 135 140
Gln Arg Val Pro Ala Arg Leu Ser Gly Ile Asp Thr Ala Gly Leu Asp
145 150 155 160
Ala His Val Pro Ala Val Arg Ala Leu Ala Trp Leu Asp Arg Gly
165 170 175

<210> 54
<211> 144

<212> PRT

<213> Mycobacterium vaccae

<400> 54

```

Gly Pro Gly Pro Arg Asn Ser Lys Phe Glu Val Val Thr Gly Met Ala
 1           5           10           15
Phe Ala Ala Phe Ala Asp Ala Pro Ile Asp Val Ala Val Val Glu Val
          20           25           30
Gly Leu Gly Gly Arg Trp Asp Ala Thr Asn Val Val Asn Ala Pro Val
          35           40           45
Ala Val Ile Thr Pro Ile Gly Val Asp His Thr Asp Tyr Leu Gly Asp
          50           55           60
Thr Ile Ala Glu Ile Ala Gly Glu Lys Ala Gly Asn His His Pro Pro
65           70           75           80
Ala Asp Asp Leu Val Pro Thr Asp Thr Val Ala Val Leu Ala Arg Gln
          85           90           95
Val Pro Glu Ala Asn Glu Val Leu Leu Ala Gln Ala Val Arg Ser Asp
          100          105          110
Ala Ala Val Ala Arg Glu Asp Ser Glu Cys Ala Val Leu Gly Arg Gln
          115          120          125
Val Ala Ile Gly Gly Ser Cys Ser Gly Cys Arg Gly Ser Val Ala Ser
          130          135          140

```

<210> 55

<211> 145

<212> PRT

<213> Mycobacterium vaccae

<400> 55

```

Asp Pro Thr Pro Ala Pro Ala Ala Ala Ser Trp Tyr Gly His Ser Ser
 1           5           10           15
Val Leu Ile Glu Val Asp Gly Tyr Arg Val Leu Ala Asp Pro Val Trp
          20           25           30
Ser Asn Arg Cys Ser Pro Ser Arg Ala Val Gly Pro Gln Arg Met His
          35           40           45
Asp Val Pro Val Pro Leu Glu Ala Leu Pro Ala Val Asp Ala Val Val
          50           55           60
Ile Ser Asn Asp His Tyr Asp His Leu Asp Ile Asp Thr Ile Val Ala
65           70           75           80
Leu Ala His Thr Gln Arg Ala Pro Phe Val Val Pro Leu Gly Ile Gly
          85           90           95
Ala His Leu Arg Lys Trp Gly Val Pro Glu Ala Arg Ile Val Glu Leu
          100          105          110
Asp Trp His Glu Ala His Arg Ile Asp Asp Leu Thr Leu Val Cys Thr
          115          120          125
Pro Ala Arg His Phe Ser Gly Arg Leu Phe Ser Arg Asp Ser Thr Leu
          130          135          140
Trp
145

```

<210> 56

<211> 10

<212> PRT

<213> Mycobacterium vaccae

<220>

<221> UNSURE

<222> (1)...(1)

<223> Residue can be either Gly, Ile, Leu or Val

<221> UNSURE

<222> (2)...(2)

<223> Residue can be either Ile, Leu, Gly, or Ala

<221> UNSURE

<222> (5)...(5)

<221> UNSURE

<222> (9)...(9)

<400> 56

Xaa Xaa Ala Pro Xaa Gly Asp Ala Xaa Arg

1

5

10

<210> 57

<211> 8

<212> PRT

<213> Mycobacterium vaccae

<220>

<221> UNSURE

<222> (7)...(7)

<223> Residue can be either Ile or Leu

<400> 57

Pro Glu Ala Glu Ala Asn Xaa Arg

1

5

<210> 58

<211> 11

<212> PRT

<213> Mycobacterium vaccae

<220>

<221> UNSURE

<222> (4)...(4)

<223> Residue can be either Gln or Gly

<221> UNSURE

<222> (5)...(5)

<223> Residue can be either Gly or Gln

<400> 58

Thr Ala Asn Xaa Xaa Glu Tyr Tyr Asp Asn Arg

1

5

10

<210> 59
 <211> 34
 <212> PRT
 <213> Mycobacterium vaccae

<400> 59
 Asn Ser Pro Arg Ala Glu Ala Glu Ala Asn Leu Arg Gly Tyr Phe Thr
 1 5 10 15
 Ala Asn Pro Ala Glu Tyr Tyr Asp Leu Arg Gly Ile Leu Ala Pro Ile
 20 25 30
 Gly Asp

<210> 60
 <211> 20
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Made in a lab

<400> 60
 ccggtgggccc cgggctgcgc 20

<210> 61
 <211> 20
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Made in a lab

<400> 61
 tggccggcca ccacgtggta 20

<210> 62
 <211> 313
 <212> DNA
 <213> Mycobacterium vaccae

<400> 62
 gccggtgggc ccgggctgcg cggaatacgc ggcagccaat cccactgggc cggcctcggt 60
 gcagggaatg tcgcaggacc cggtcgcggt ggcggcctcg aacaatccgg agttgacaac 120
 gctgtacggc tgcactgtcg ggccagctca atccgcaagt aaacctgggtg gacaccctca 180
 acagcgggtca gtacacgggtg ttcgcaccga ccaacgcggc atttagcaag ctgccggcat 240
 ccacgatcga cgagctcaag accaattcgt cactgctgac cagcatcctg acctaccacg 300
 tgggtggcgg cca 313

<210> 63
 <211> 18
 <212> PRT
 <213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (7)...(17)

<400> 63
Glu Pro Ala Gly Pro Leu Pro Xaa Tyr Asn Glu Arg Leu His Thr Leu
1 5 10 15
Xaa Gln

<210> 64
<211> 25
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (21)...(21)

<400> 64
Gly Leu Asp Asn Glu Leu Ser Leu Val Asp Gly Gln Gly Arg Thr Leu
1 5 10 15
Thr Val Gln Gln Xaa Asp Thr Phe Leu
20 25

<210> 65
<211> 26
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (3)...(3)

<221> UNSURE
<222> (21)...(22)

<221> UNSURE
<222> (24)...(24)

<400> 65
Asp Pro Xaa Pro Asp Ile Glu Val Glu Phe Ala Arg Gly Thr Gly Ala
1 5 10 15
Glu Pro Gly Leu Xaa Xaa Val Xaa Asp Ala
20 25

<210> 66
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 66
accgccctcg agttctcccg gccaggtctg cc 32

<210> 67
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 67
aagcacgagc tcagtctctt ccacgcggac gt 32

<210> 68
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 68
catggatcca ttctcccggc ccggtcttcc 30

<210> 69
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 69
tttgaattct aggcggtggc ctgagc 26

<210> 70
<211> 161
<212> PRT
<213> Mycobacterium vaccae

<400> 70
Ser Gly Trp Asp Ile Asn Thr Ala Ala Phe Glu Trp Tyr Val Asp Ser
1 5 10 15
Gly Leu Ala Val Ile Met Pro Val Gly Gly Gln Ser Ser Phe Tyr Ser
20 25 30
Asp Trp Tyr Ser Pro Ala Cys Gly Lys Ala Gly Cys Gln Thr Tyr Lys
35 40 45
Trp Glu Thr Phe Leu Thr Gln Glu Leu Pro Ala Tyr Leu Ala Ala Asn
50 55 60
Lys Gly Val Asp Pro Asn Arg Asn Ala Ala Val Gly Leu Ser Met Ala
65 70 75 80

Gly	Ser	Ala	Ala	Leu	Thr	Leu	Ala	Ile	Tyr	His	Pro	Gln	Gln	Phe	Gln
				85					90					95	
Tyr	Ala	Gly	Ser	Leu	Ser	Gly	Tyr	Leu	Asn	Pro	Ser	Glu	Gly	Trp	Trp
			100					105					110		
Pro	Met	Leu	Ile	Asn	Ile	Ser	Met	Gly	Asp	Ala	Gly	Gly	Tyr	Lys	Ala
		115					120					125			
Asn	Asp	Met	Trp	Gly	Arg	Thr	Glu	Asp	Pro	Ser	Ser	Ala	Trp	Lys	Arg
	130					135					140				
Asn	Asp	Pro	Met	Val	Asn	Ile	Gly	Lys	Leu	Val	Ala	Asn	Asn	Thr	Pro
145					150				155						160
Leu															

<210> 71
 <211> 33
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Made in a lab

<400> 71
 gagagactcg agaacgccca ggaagggcac cag

33

<210> 72
 <211> 32
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Made in a lab

<400> 72
 gagagactcg agtgactcac cactgaccga gc

32

<210> 73
 <211> 20
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Made in a lab

<221> unsure
 <222> (3)...(3)

<221> unsure
 <222> (6)...(6)

<221> unsure
 <222> (9)...(9)

<221> unsure

<222> (15) ... (15)

<400> 73

ggngcngcnc argcngarcc

20

<210> 74

<211> 825

<212> DNA

<213> Mycobacterium vaccae

<400> 74

ttggatccca	ctcccgcgcc	ggcggcgccc	agctggtagc	gccattccag	cgtgctgac	60
gaggtcgacg	gctaccgcgt	gctggccgac	ccggtgtgga	gcaacagatg	ttcgccctca	120
cgggcggtcg	gaccgcagcg	catgcacgac	gtcccggtgc	cgctggaggc	gcttcccgcc	180
gtggacgcgg	tggtgatcag	ccacgaccac	tacgaccacc	tcgacatcga	caccatcgtc	240
gcgttggcgc	acaccacgcg	ggccccgttc	gtggtgccgt	tgggcacatg	cgcacacctg	300
cgcaagtggg	gcgtccccga	ggcgcggatc	gtcgagttgg	actggcacga	agcccaccgc	360
atagacgacc	tgacgctggt	ctgcaccccc	gcccggcact	tctccggacg	gttgttctcc	420
cgcgactcga	cgctgtgggc	gtcgtgggtg	gtcaccggct	cgtcgcacaa	ggcgttcttc	480
ggtggcgaca	ccggatacac	gaagagcttc	gcccagatcg	gcgacgagta	cggtcctgtc	540
gatctgaccc	tgctgcccgt	cggggcctac	catcccgctg	tcgccgacat	ccacatgaac	600
cccgaggagg	cggtgcgcgc	ccatctggac	ctgaccgagg	tggacaacag	cctgatgggtg	660
cccatccact	gggcgacatt	ccgcctcgcc	ccgcacccgt	ggtccgagcc	cgccgaacgc	720
ctgctgaccg	ctgccgacgc	cgagcgggta	cgcccgaccg	tgccgattcc	cggtcagcgg	780
gtggaccccg	agtcgacgtt	cgaccctgtg	tggcggttct	gaacc		825

<210> 75

<211> 273

<212> PRT

<213> Mycobacterium vaccae

<400> 75

Leu	Asp	Pro	Thr	Pro	Ala	Pro	Ala	Ala	Ala	Ser	Trp	Tyr	Gly	His	Ser
1				5				10						15	
Ser	Val	Leu	Ile	Glu	Val	Asp	Gly	Tyr	Arg	Val	Leu	Ala	Asp	Pro	Val
			20					25					30		
Trp	Ser	Asn	Arg	Cys	Ser	Pro	Ser	Arg	Ala	Val	Gly	Pro	Gln	Arg	Met
			35					40					45		
His	Asp	Val	Pro	Val	Pro	Leu	Glu	Ala	Leu	Pro	Ala	Val	Asp	Ala	Val
			50					55					60		
Val	Ile	Ser	His	Asp	His	Tyr	Asp	His	Leu	Asp	Ile	Asp	Thr	Ile	Val
			65			70				75				80	
Ala	Leu	Ala	His	Thr	Gln	Arg	Ala	Pro	Phe	Val	Val	Pro	Leu	Gly	Ile
				85					90					95	
Gly	Ala	His	Leu	Arg	Lys	Trp	Gly	Val	Pro	Glu	Ala	Arg	Ile	Val	Glu
			100					105					110		
Leu	Asp	Trp	His	Glu	Ala	His	Arg	Ile	Asp	Asp	Leu	Thr	Leu	Val	Cys
			115				120					125			
Thr	Pro	Ala	Arg	His	Phe	Ser	Gly	Arg	Leu	Phe	Ser	Arg	Asp	Ser	Thr
			130				135					140			
Leu	Trp	Ala	Ser	Trp	Val	Val	Thr	Gly	Ser	Ser	His	Lys	Ala	Phe	Phe
			145			150				155				160	
Gly	Gly	Asp	Thr	Gly	Tyr	Thr	Lys	Ser	Phe	Ala	Glu	Ile	Gly	Asp	Glu

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                165                170                175
Tyr Gly Pro Phe Asp Leu Thr Leu Leu Pro Ile Gly Ala Tyr His Pro
                180                185                190
Ala Phe Ala Asp Ile His Met Asn Pro Glu Glu Ala Val Arg Ala His
                195                200                205
Leu Asp Leu Thr Glu Val Asp Asn Ser Leu Met Val Pro Ile His Trp
                210                215                220
Ala Thr Phe Arg Leu Ala Pro His Pro Trp Ser Glu Pro Ala Glu Arg
225                230                235                240
Leu Leu Thr Ala Ala Asp Ala Glu Arg Val Arg Leu Thr Val Pro Ile
                245                250                255
Pro Gly Gln Arg Val Asp Pro Glu Ser Thr Phe Asp Pro Trp Trp Arg
                260                265                270
Phe

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<210> 76
<211> 10
<212> PRT
<213> Mycobacterium vaccae

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<400> 76
Ala Lys Thr Ile Ala Tyr Asp Glu Glu Ala
 1                5                10

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<210> 77
<211> 337
<212> DNA
<213> Mycobacterium vaccae

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<400> 77
gatccctaca tcctgctggt cagctccaag gtgtcgaccg tcaaggatct gctcccgtcg      60
ctggagaagg tcatccaggc cggcaagccg ctgctgatca tcgccgagga cgtcgagggc      120
gaggccctgt ccacgctggt ggtcaacaag atccgcggca ctttcaagtc cgtcgccgtc      180
aaggctccgg gcttcgggtga ccgccgcaag gcgatgctgc aggacatggc catcctcacc      240
ggtggtcagg tcgtcagcga aagagtcggg ctgtccctgg agaccgccga cgtctcgctg      300
ctgggccagg cccgcaaggt cgtcgtcacc aaggaca                                337

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<210> 78
<211> 112
<212> PRT
<213> Mycobacterium vaccae

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<400> 78
Asp Pro Tyr Ile Leu Leu Val Ser Ser Lys Val Ser Thr Val Lys Asp
 1                5                10                15
Leu Leu Pro Leu Leu Glu Lys Val Ile Gln Ala Gly Lys Pro Leu Leu
                20                25                30
Ile Ile Ala Glu Asp Val Glu Gly Glu Ala Leu Ser Thr Leu Val Val
                35                40                45
Asn Lys Ile Arg Gly Thr Phe Lys Ser Val Ala Val Lys Ala Pro Gly
 50                55                60
Phe Gly Asp Arg Arg Lys Ala Met Leu Gln Asp Met Ala Ile Leu Thr

```

65		70		75		80									
Gly	Gly	Gln	Val	Val	Ser	Glu	Arg	Val	Gly	Leu	Ser	Leu	Glu	Thr	Ala
			85						90					95	
Asp	Val	Ser	Leu	Leu	Gly	Gln	Ala	Arg	Lys	Val	Val	Val	Thr	Lys	Asp
			100						105					110	

<210> 79

<211> 360

<212> DNA

<213> Mycobacterium vaccae

<400> 79

ccgtacgaga	agatcggcgc	tgagctgggc	aaagaggtcg	ccaagaagac	cgacgacgtc	60
gcgggcgacg	gcaccaccac	cgccaccgtg	ctcgctcagg	ctctgggttcg	cgaaggcctg	120
cgcaacgtcg	cagccggcgc	caaccogctc	ggcctcaagc	gtggcatcga	gaaggctgtc	180
gaggctgtca	cccagtcgct	gctgaagtcg	gccaaggagg	tcgagaccaa	ggagcagatt	240
tctgccaccg	cggcgatctc	cgcgggcgac	accagatcgc	gcgagctcat	cgccgaggcc	300
atggacaagg	tcggcaacga	gggtgtcatc	accgtcgagg	agtcgaacac	cttcggcctg	360

<210> 80

<211> 120

<212> PRT

<213> Mycobacterium vaccae

<400> 80

Pro	Tyr	Glu	Lys	Ile	Gly	Ala	Glu	Leu	Val	Lys	Glu	Val	Ala	Lys	Lys
1				5					10					15	
Thr	Asp	Asp	Val	Ala	Gly	Asp	Gly	Thr	Thr	Thr	Ala	Thr	Val	Leu	Ala
			20					25					30		
Gln	Ala	Leu	Val	Arg	Glu	Gly	Leu	Arg	Asn	Val	Ala	Ala	Gly	Ala	Asn
			35				40					45			
Pro	Leu	Gly	Leu	Lys	Arg	Gly	Ile	Glu	Lys	Ala	Val	Glu	Ala	Val	Thr
			50			55					60				
Gln	Ser	Leu	Leu	Lys	Ser	Ala	Lys	Glu	Val	Glu	Thr	Lys	Glu	Gln	Ile
65					70				75					80	
Ser	Ala	Thr	Ala	Ala	Ile	Ser	Ala	Gly	Asp	Thr	Gln	Ile	Gly	Glu	Leu
			85						90					95	
Ile	Ala	Glu	Ala	Met	Asp	Lys	Val	Gly	Asn	Glu	Gly	Val	Ile	Thr	Val
			100					105					110		
Glu	Glu	Ser	Asn	Thr	Phe	Gly	Leu								
			115				120								

<210> 81

<211> 43

<212> DNA

<213> Artificial Sequence

<220>

<223> Made in a lab

<400> 81

actgacgctg aggagcgaaa gcgtggggag cgaacaggat tag

43

<210> 82
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 82
cgacaaggaa cttcgctacc ttaggaccgt catagttacg ggc 43

<210> 83
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 83
aaaaaaaaaa aaaaaaaaaa 20

<210> 84
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 84
ggaaggaagc ggccgctttt tttttttttt t 31

<210> 85
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 85
gagagagagc ccgggcatgc tsctsctsct s 31

<210> 86
<211> 238
<212> DNA
<213> Mycobacterium vaccae

<400> 86
ctcgatgaac cgctcggagc gctcgacctg aagctgcgcc acgtcatgca gttcgagctc 60
aagcgcaccc agcgggaggt cgggatcacg ttcattctacg tgaccacga ccaggaagag 120
gcgctcacga tgagtgaccg catcgcggtg atgaacgccg gcaacgtcga acagatcggc 180

agccccgaccg agatctacga ccgtccccgcg acggtgttcg tcgccagctt catcgaat

238

<210> 87

<211> 79

<212> PRT

<213> Mycobacterium vaccae

<400> 87

Leu	Asp	Glu	Pro	Leu	Gly	Ala	Leu	Asp	Leu	Lys	Leu	Arg	His	Val	Met
1			5					10					15		
Gln	Phe	Glu	Leu	Lys	Arg	Ile	Gln	Arg	Glu	Val	Gly	Ile	Thr	Phe	Ile
			20				25					30			
Tyr	Val	Thr	His	Asp	Gln	Glu	Glu	Ala	Leu	Thr	Met	Ser	Asp	Arg	Ile
	35					40				45					
Ala	Val	Met	Asn	Ala	Gly	Asn	Val	Glu	Gln	Ile	Gly	Ser	Pro	Thr	Glu
	50				55					60					
Ile	Tyr	Asp	Arg	Pro	Ala	Thr	Val	Phe	Val	Ala	Ser	Phe	Ile	Glu	
65					70					75					

<210> 88

<211> 1518

<212> DNA

<213> Mycobacterium vaccae

<400> 88

cactcgccat	gggtgttaca	ataccccacc	agttcctcga	agtaaacgaa	cagaaccgtg	60
acatccagct	gagaaaatat	tcacagcgac	gaagcccggc	cgatgcctga	tgggggtccgg	120
catcagtaca	gcgcgctttc	ctgcgcggat	tctattgtcg	agtcgggggt	gtgacgaagg	180
aatccattgt	cgaaatgtaa	attcgttgcg	gaatcacttg	catagggtccg	tcagatccgc	240
gaaggtttac	cccacagcca	cgacggctgt	ccccgaggag	gacctgccct	gaccggcaca	300
cacatcaccg	ctgcagaacc	tcgagaacag	acggcggatt	ccgcggcacc	gccaagggc	360
gcgcgggtga	tcgagatcga	ccatgtcacg	aagcgcttcg	gcgactacct	ggccgtcgcg	420
gacgcagact	tctccatcgc	gcccggggag	ttcttctcca	tgctcggccc	gtccgggtgt	480
gggaagacga	ccacgttgcg	catgatcgcg	ggattcgaga	ccccgactga	aggggcgatc	540
gcgctcgaag	gcgcccagct	gtcgaggacc	ccaccaaca	agcgcaacgt	caacacgggtg	600
ttccagcact	acgcgctgtt	cccgcacatg	acggtctggg	acaacgtcgc	gtacggccccg	660
cgcagcaaga	aactcggcaa	aggcgaggtc	cgcaagcgcg	tcgacgagct	gctggagatc	720
gtccgggtga	ccgaatttgc	cgagcgcagg	cccgcaccagc	tgtccggcgg	gcagcagcag	780
cgggtggcgt	tggcccgggc	actggtgaac	taccccagcg	cgctgctgct	cgatgaaccg	840
ctcggagcgc	tcgacctgaa	gctgcgccac	gtcatgcagt	tcgagctcaa	gcgcatccag	900
cgggaggtcg	ggatcacgtt	catctacgtg	acccacgacc	aggaagaggc	gtcacgatg	960
agtgaccgca	tcgcggtgat	gaacgccggc	aacgtcgaac	agatcggcag	cccgaccgag	1020
atctacgacc	gtcccgcgac	ggtgttcgtc	gccagcttca	tcggacaggc	caacctctgg	1080
gcggggccggt	gcaccggccg	ctccaaccgc	gattacgtcg	agatcgacgt	tctcggctcg	1140
acgtgaagg	cacgcccggg	cgagaccacg	atcgagcccc	gcgggcacgc	caccctgatg	1200
gtgcgtccgg	aacgcacccg	ggtcaccccc	ggctcccagg	acgcgccgac	cggtgacgtc	1260
gcctgcgtgc	gtgccaccgt	caccgacctg	accttccaag	gtccggtggt	gcggctctcg	1320
ctggccgctc	cggacgactc	gaccgtgacg	gcccacgtcg	gccccgagca	ggatctgccg	1380
ctgctgcgcc	ccggcgacga	cgtgtacgtc	agctgggcac	cggaagcctc	cctggtgctt	1440
cccggcgacg	acatccccac	caccgaggac	ctcgaagaga	tgctcgacga	ctcctgagtc	1500
acgcttcccc	attgccga					1518

<210> 89

<211> 376

<212> PRT

<213> Mycobacterium vaccae

<400> 89

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Val Ile Glu Ile Asp His Val Thr Lys Arg Phe Gly Asp Tyr Leu Ala
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Val Ala Asp Ala Asp Phe Ser Ile Ala Pro Gly Glu Phe Phe Ser Met
      20           25           30
Leu Gly Pro Ser Gly Cys Gly Lys Thr Thr Thr Leu Arg Met Ile Ala
      35           40           45
Gly Phe Glu Thr Pro Thr Glu Gly Ala Ile Arg Leu Glu Gly Ala Asp
      50           55           60
Val Ser Arg Thr Pro Pro Asn Lys Arg Asn Val Asn Thr Val Phe Gln
      65           70           75           80
His Tyr Ala Leu Phe Pro His Met Thr Val Trp Asp Asn Val Ala Tyr
      85           90           95
Gly Pro Arg Ser Lys Lys Leu Gly Lys Gly Glu Val Arg Lys Arg Val
      100          105          110
Asp Glu Leu Leu Glu Ile Val Arg Leu Thr Glu Phe Ala Glu Arg Arg
      115          120          125
Pro Ala Gln Leu Ser Gly Gly Gln Gln Gln Arg Val Ala Leu Ala Arg
      130          135          140
Ala Leu Val Asn Tyr Pro Ser Ala Leu Leu Leu Asp Glu Pro Leu Gly
      145          150          155          160
Ala Leu Asp Leu Lys Leu Arg His Val Met Gln Phe Glu Leu Lys Arg
      165          170          175
Ile Gln Arg Glu Val Gly Ile Thr Phe Ile Tyr Val Thr His Asp Gln
      180          185          190
Glu Glu Ala Leu Thr Met Ser Asp Arg Ile Ala Val Met Asn Ala Gly
      195          200          205
Asn Val Glu Gln Ile Gly Ser Pro Thr Glu Ile Tyr Asp Arg Pro Ala
      210          215          220
Thr Val Phe Val Ala Ser Phe Ile Gly Gln Ala Asn Leu Trp Ala Gly
      225          230          235          240
Arg Cys Thr Gly Arg Ser Asn Arg Asp Tyr Val Glu Ile Asp Val Leu
      245          250          255
Gly Ser Thr Leu Lys Ala Arg Pro Gly Glu Thr Thr Ile Glu Pro Gly
      260          265          270
Gly His Ala Thr Leu Met Val Arg Pro Glu Arg Ile Arg Val Thr Pro
      275          280          285
Gly Ser Gln Asp Ala Pro Thr Gly Asp Val Ala Cys Val Arg Ala Thr
      290          295          300
Val Thr Asp Leu Thr Phe Gln Gly Pro Val Val Arg Leu Ser Leu Ala
      305          310          315          320
Ala Pro Asp Asp Ser Thr Val Ile Ala His Val Gly Pro Glu Gln Asp
      325          330          335
Leu Pro Leu Leu Arg Pro Gly Asp Asp Val Tyr Val Ser Trp Ala Pro
      340          345          350
Glu Ala Ser Leu Val Leu Pro Gly Asp Asp Ile Pro Thr Thr Glu Asp
      355          360          365
Leu Glu Glu Met Leu Asp Asp Ser
      370          375

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<210> 90
 <211> 33
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Made in a lab

<400> 90
 gagagactcg aggtgatcga gatcgaccat gtc

33

<210> 91
 <211> 31
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Made in a lab

<400> 91
 agagactcga gcaatcgga agcgtgactc a

31

<210> 92
 <211> 323
 <212> DNA
 <213> Mycobacterium vaccae

<400> 92
 gtcgactaca aagaagactt caacgacaac gagcagtggt tcgccaaggt caaggagccg 60
 ttgtcgcgca agcaggacat aggcgccgac ctggtgatcc ccaccgagtt catggccgcg 120
 cgcgtcaagg gcctgggatg gctcaatgag atcagcgaag ccggcgtgcc caatcgcaag 180
 aatctgcgtc aggacctgtt ggactcgagc atcgacgagg gccgcaagtt caccgcgcgcg 240
 tacatgaccg gcatggtcgg tctcgctac aacaaggcag ccaccggacg cgatatccgc 300
 accatcgacg acctctggga tcc 323

<210> 93
 <211> 1341
 <212> DNA
 <213> Mycobacterium vaccae

<400> 93
 cccaccccc ttccctggag ccgacgaaag gcaccgcgac atgtcccgtg acatcgatcc 60
 ccacctgctg gcccgaaatga ccgcacgccg caccttgctg cgcgcgttca tcggcggtgg 120
 cgccgcggcc gccgcggggc tgacctcgg ttcgtcgttc ctggcggtcg gcgggtccga 180
 cagtgggacc tcgagcacca cgtcacagga cagcggtccc gccagcggtg ccctgcgcgt 240
 ctccaactgg ccgtctata tggccgacgg ttcatcgca gcgttcocaga ccgcctcggg 300
 catcacggtc gactacaaag aagacttcaa cgacaacgag cagtgggtcg ccaaggtcaa 360
 ggagcgttg tcgcgcaagc aggacatagg cgccgacctg gtgatcccca ccgagttcat 420
 ggccgcgcgc gtcaagggcc tgggatggct caatgagatc agcgaagccg gcgtgcccga 480
 tcgcaagaat ctgcgtcagg acctgttgga ctcgagcatc gacgagggcc gcaagttcac 540
 cgcgcgttac atgaccggca tggtcggtct cgcctacaac aaggcagcca ccggacgcga 600
 tatccgcacc atcgacgacc tctgggatcc cgcgttcaag ggccgcgtca gtctgttctc 660


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cgacgtccag gacggcctcg gcatgatcat gctctcgcag ggcaactcgc cggagaatcc 720
gaccaccgag tccattcagc aggcgggtcga tctgggtccgc gaacagaacg acaggggggtc 780
agatccggtcg cttcacccggc aacgactacg ccgacgacct ggccgcagaa acatcgccat 840
cgcgcgaggcg tactccgggtg acgtcgtgca gctgcaggcg gacaaccccg atctgcagtt 900
catcgttccc gaatccggcg gcgactgggtt cgctcgacacg atgggtgatcc cgtacaccac 960
gcagaaccag aaggccggcg aggcgtggat cgactacatc tacgaccgag ccaactacgc 1020
caagctggtc gcgttcaccc agttcgtgcc cgcactctcg gacatgaccg acgaactcgc 1080
caaggtcgat cctgcatcgg cggagaaccc gctgatcaac ccgtcggccg aggtgcaggc 1140
gaacctgaag tcgtggggcgg cactgaccga cgagcagacg caggagttca acaactgcgta 1200
cgccgccgtc accggcggct gacgcggtgg tagtgccgat gcgaggggca taaatggccc 1260
tgcggaacgc agggacataa atggccgggtg tcgccaccag cagccgtcag cggacaaggt 1320
cgctccgtat ctgatgggtcc t 1341

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<210> 94

<211> 393

<212> PRT

<213> Mycobacterium vaccae

<400> 94

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Met Ser Arg Asp Ile Asp Pro His Leu Leu Ala Arg Met Thr Ala Arg
1          5          10          15
Arg Thr Leu Arg Arg Arg Phe Ile Gly Gly Gly Ala Ala Ala Ala
20          25          30
Gly Leu Thr Leu Gly Ser Ser Phe Leu Ala Ala Cys Gly Ser Asp Ser
35          40          45
Gly Thr Ser Ser Thr Thr Ser Gln Asp Ser Gly Pro Ala Ser Gly Ala
50          55          60
Leu Arg Val Ser Asn Trp Pro Leu Tyr Met Ala Asp Gly Phe Ile Ala
65          70          75          80
Ala Phe Gln Thr Ala Ser Gly Ile Thr Val Asp Tyr Lys Glu Asp Phe
85          90          95
Asn Asp Asn Glu Gln Trp Phe Ala Lys Val Lys Glu Pro Leu Ser Arg
100         105         110
Lys Gln Asp Ile Gly Ala Asp Leu Val Ile Pro Thr Glu Phe Met Ala
115         120         125
Ala Arg Val Lys Gly Leu Gly Trp Leu Asn Glu Ile Ser Glu Ala Gly
130         135         140
Val Pro Asn Arg Lys Asn Leu Arg Gln Asp Leu Leu Asp Ser Ser Ile
145         150         155         160
Asp Glu Gly Arg Lys Phe Thr Ala Pro Tyr Met Thr Gly Met Val Gly
165         170         175
Leu Ala Tyr Asn Lys Ala Ala Thr Gly Arg Asp Ile Arg Thr Ile Asp
180         185         190
Asp Leu Trp Asp Pro Ala Phe Lys Gly Arg Val Ser Leu Phe Ser Asp
195         200         205
Val Gln Asp Gly Leu Gly Met Ile Met Leu Ser Gln Gly Asn Ser Pro
210         215         220
Glu Asn Pro Thr Thr Glu Ser Ile Gln Gln Ala Val Asp Leu Val Arg
225         230         235         240
Glu Gln Asn Asp Arg Gly Ser Asp Pro Ser Leu His Arg Gln Arg Leu
245         250         255
Arg Arg Arg Pro Gly Arg Arg Asn Ile Ala Ile Ala Gln Ala Tyr Ser
260         265         270

```

Gly Asp Val Val Gln Leu Gln Ala Asp Asn Pro Asp Leu Gln Phe Ile
 275 280 285
 Val Pro Glu Ser Gly Gly Asp Trp Phe Val Asp Thr Met Val Ile Pro
 290 295 300
 Tyr Thr Thr Gln Asn Gln Lys Ala Ala Glu Ala Trp Ile Asp Tyr Ile
 305 310 315 320
 Tyr Asp Arg Ala Asn Tyr Ala Lys Leu Val Ala Phe Thr Gln Phe Val
 325 330 335
 Pro Ala Leu Ser Asp Met Thr Asp Glu Leu Ala Lys Val Asp Pro Ala
 340 345 350
 Ser Ala Glu Asn Pro Leu Ile Asn Pro Ser Ala Glu Val Gln Ala Asn
 355 360 365
 Leu Lys Ser Trp Ala Ala Leu Thr Asp Glu Gln Thr Gln Glu Phe Asn
 370 375 380
 Thr Ala Tyr Ala Ala Val Thr Gly Gly
 385 390

<210> 95

<211> 22

<212> DNA

<213> Mycobacterium vaccae

<400> 95

atgtcccggtg acatcgatcc cc

22

<210> 96

<211> 21

<212> DNA

<213> Mycobacterium vaccae

<400> 96

atcggcacta ccaccgcgtc a

21

<210> 97

<211> 861

<212> DNA

<213> Mycobacterium vaccae

<400> 97

gccggcgctc	gcataatctcg	cgatcttctt	ccgtgggtgcc	gttcttctcg	ctggcacgca	60
cctcgttgtc	ggagaccggc	ggctcggtgt	tcatgccgac	gctgacgttc	gcctgggact	120
tcggcaacta	cgtcgacgcg	ttcacgatgt	accacgagca	gatcttccgc	tcgttcggct	180
acgcgttcgt	cgccacggtg	ctgtgcctgt	tgetggcggt	cccgtgggcc	tacgtcatcg	240
cgttcaaggc	cggccgggttc	aagaacctga	tectggggct	ggtgacccgt	ccgttcttcg	300
tcacgttcct	gatccgcacc	attgcgtgga	agacgatcct	ggccgacgaa	ggctgggtgg	360
tcaccgcgct	gggcgccatc	gggctgctgc	ctgacgaggg	ccggctgctg	tccaccagct	420
gggcgggtcat	cggcggtctg	acctacaact	ggatcatctt	catgacccgt	ccgctgtacg	480
tcagccctgga	gaagatcgac	ccgcgtctgc	tggaggcctc	ccaggacctc	tactcgctcg	540
cgcgcgcgag	cttcggcaag	gtgatcctgc	cgatggcgat	gcccgggggtg	ctggccggga	600
gcatgctggt	gttcatcccg	gccgtcggcg	acttcatcaa	cgcgcgactat	ctcggcagta	660
cccagaccac	catgatcggc	aacgtgatcc	agaagcagtt	cctgggtcgtc	aaggactatc	720
cggcgggcggc	cgcgctgagt	ctggggctga	tggtgctgat	cctgatccggc	gtgctcctct	780
acacacgggc	gctggggttcg	gaggatctgg	tatgaccacc	caggcaggcg	ccgcactggc	840

caccgccgcc cagcaggatc c

861

<210> 98

<211> 259

<212> PRT

<213> Mycobacterium vaccae

<400> 98

Val	Val	Pro	Phe	Ser	Leu	Ala	Arg	Thr	Ser	Leu	Ser	Glu	Thr	Gly
1			5					10					15	
Gly	Ser	Val	Phe	Met	Pro	Thr	Leu	Thr	Phe	Ala	Trp	Asp	Phe	Gly
			20				25					30		Asn
Tyr	Val	Asp	Ala	Phe	Thr	Met	Tyr	His	Glu	Gln	Ile	Phe	Arg	Ser
		35					40					45		Phe
Gly	Tyr	Ala	Phe	Val	Ala	Thr	Val	Leu	Cys	Leu	Leu	Leu	Ala	Phe
	50					55				60				Pro
Leu	Ala	Tyr	Val	Ile	Ala	Phe	Lys	Ala	Gly	Arg	Phe	Lys	Asn	Leu
65				70					75					80
Leu	Gly	Leu	Val	Ile	Leu	Pro	Phe	Phe	Val	Thr	Phe	Leu	Ile	Arg
			85					90						95
Ile	Ala	Trp	Thr	Ile	Leu	Ala	Asp	Glu	Gly	Trp	Val	Val	Thr	Ala
			100					105					110	Leu
Gly	Ala	Ile	Gly	Leu	Leu	Pro	Asp	Glu	Gly	Arg	Leu	Leu	Ser	Thr
	115					120					125			Ser
Trp	Ala	Val	Ile	Gly	Gly	Leu	Thr	Tyr	Asn	Trp	Ile	Ile	Phe	Met
	130					135					140			Ile
Leu	Pro	Leu	Tyr	Val	Ser	Leu	Glu	Lys	Ile	Asp	Pro	Arg	Leu	Leu
145				150					155					160
Ala	Ser	Gln	Asp	Leu	Tyr	Ser	Ser	Ala	Pro	Arg	Ser	Phe	Gly	Lys
			165					170						175
Ile	Leu	Pro	Met	Ala	Met	Pro	Gly	Val	Leu	Ala	Gly	Ser	Met	Leu
			180				185						190	Val
Phe	Ile	Pro	Ala	Val	Gly	Asp	Phe	Ile	Asn	Ala	Asp	Tyr	Leu	Gly
	195					200						205		Ser
Thr	Gln	Thr	Thr	Met	Ile	Gly	Asn	Val	Ile	Gln	Lys	Gln	Phe	Leu
	210					215						220		Val
Val	Lys	Asp	Tyr	Pro	Ala	Ala	Ala	Ala	Leu	Ser	Leu	Gly	Leu	Met
225				230					235					240
Leu	Ile	Leu	Ile	Gly	Val	Leu	Leu	Tyr	Thr	Arg	Ala	Leu	Gly	Ser
			245					250						255
Asp	Leu	Val												

<210> 99

<211> 277

<212> DNA

<213> Mycobacterium vaccae

<400> 99

gtaatctttg	ctggagcccg	tacgccggta	ggcaaaactca	tgggttcgct	caaggacttc	60
aagggcagcg	atctcggtgc	cgtggcgatc	aagggcgccc	tggagaaagc	cttccccggc	120
gtcgacgacc	ctgctcgtct	cgtcgagtac	gtgatcatgg	gccaagtgct	ctccgccggc	180
gccggccaga	tgcccgcccg	ccaggccgcc	gtcgccggccg	gcattcccg	ggacgtcgcc	240

tcgctgacga tcaacaagat gtgcctgtcg ggcacgcg

277

<210> 100
 <211> 92
 <212> PRT
 <213> Mycobacterium vaccae

<400> 100
 Val Ile Phe Ala Gly Ala Arg Thr Pro Val Gly Lys Leu Met Gly Ser
 1 5 10 15
 Leu Lys Asp Phe Lys Gly Ser Asp Leu Gly Ala Val Ala Ile Lys Gly
 20 25 30
 Ala Leu Glu Lys Ala Phe Pro Gly Val Asp Asp Pro Ala Arg Leu Val
 35 40 45
 Glu Tyr Val Ile Met Gly Gln Val Leu Ser Ala Gly Ala Gly Gln Met
 50 55 60
 Pro Ala Arg Gln Ala Ala Val Ala Ala Gly Ile Pro Trp Asp Val Ala
 65 70 75 80
 Ser Leu Thr Ile Asn Lys Met Cys Leu Ser Gly Ile
 85 90

<210> 101
 <211> 12
 <212> PRT
 <213> Mycobacterium vaccae

<220>
 <221> UNSURE
 <222> (1)...(1)
 <223> Residue can be either Glu or Pro

<221> UNSURE
 <222> (2)...(2)
 <223> Residue can be either Pro or Glu

<221> UNSURE
 <222> (7)...(7)

<221> UNSURE
 <222> (12)...(12)

<400> 101
 Xaa Xaa Ala Asp Arg Gly Xaa Ser Lys Tyr Arg Xaa
 1 5 10

<210> 102
 <211> 24
 <212> PRT
 <213> Mycobacterium vaccae

<220>
 <221> UNSURE
 <222> (1)...(1)

<400> 102
Xaa Ile Asp Glu Ser Leu Phe Asp Ala Glu Glu Lys Met Glu Lys Ala
1 5 10 15
Val Ser Val Ala Arg Asp Ser Ala
20

<210> 103
<211> 23
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (1)...(2)

<221> UNSURE
<222> (15)...(15)

<221> UNSURE
<222> (17)...(17)

<400> 103
Xaa Xaa Ile Ala Pro Ala Thr Ser Gly Thr Leu Ser Glu Phe Xaa Ala
1 5 10 15
Xaa Lys Gly Val Thr Met Glu
20

<210> 104
<211> 15
<212> PRT
<213> Mycobacterium vaccae

<400> 104
Pro Asn Val Pro Asp Ala Phe Ala Val Leu Ala Asp Arg Val Gly
1 5 10 15

<210> 105
<211> 9
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (1)...(1)

<400> 105
Xaa Ile Arg Val Gly Val Asn Gly Phe
1 5

<210> 106
<211> 485
<212> DNA

<213> Mycobacterium vaccae

<400> 106

agcggctggg	acatcaacac	cgccgccttc	gagtgggtacg	tgcactcggg	tctcgcggtg	60
atcatgcccc	tggcggggca	gtccagcttc	tacagcgact	ggtacagccc	ggcctgcggt	120
aaggccggct	gccagacctt	caagtgggag	acgttcctga	cccaggagct	gccggcctac	180
ctcgccgcca	acaagggggt	cgacccgaac	cgcaacgcgg	ccgtcgggtct	gtccatggcc	240
ggttcggcgg	cgctgacgct	ggcgatctac	caccgcagc	agttccagta	cgccggggtcg	300
ctgtcgggct	acctgaaccc	gtccgagggg	tgggtggccga	tgtgatcaa	catctcgatg	360
ggtgacgcgg	gcggtacaa	ggccaacgac	atgtggggtc	gcaccgagga	cccgagcagc	420
gcctggaagc	gcaacgaccc	gatggtcaac	atcggcaagc	tggtcgcca	caacaccccc	480
ctctc						485

<210> 107

<211> 501

<212> DNA

<213> Mycobacterium vaccae

<220>

<221> unsure

<222> (441)...(441)

<221> unsure

<222> (450)...(450)

<400> 107

atgccggtgc	gacgtgcgcg	cagtgcgctt	gcgtccgtga	ccttcgctgc	ggccgcggtgc	60
gtgggcgctg	agggcaccgc	actggcgggc	acgccggact	ggagcggggc	ctacacgggtg	120
gtgacgttcg	cctccgacaa	actcggcacg	agtgtggccg	cccgccagcc	agaacccgac	180
ttcagcggtc	agtacacctt	cagcacgtcc	tgtgtgggca	cctgcgtggc	caccgcgtcc	240
gacggcccg	cgccgtcgaa	cccgaacgatt	ccgcagcccg	cgcgctacac	ctgggacggc	300
aggcagtg	tggtcaacta	caactggcag	tgggagtgct	tccgcggcgc	cgacgtccc	360
cgcgagtacg	ccgcgcgcg	ttcgtggtg	ttctacgcc	cgaccgcga	cgggtcgatg	420
ttcggcacct	ggcgcaccga	natcctggan	ggcctctgca	agggcacctg	gatcatgccc	480
gtcgcggcct	atccggcgta	g				501

<210> 108

<211> 180

<212> DNA

<213> Mycobacterium vaccae

<400> 108

atgaaccagc	cgcgccccga	ggccgaggcg	aacctgcggg	gctacttcac	cgccaacccg	60
gcgagtagt	acgacctg	gggcacctc	gccccgatc	gtgacgcgca	gcgcaactgc	120
aacatcaccc	tgctgccggt	agagctgcag	acggcctacg	acacgttcat	ggccgggctga	180

<210> 109

<211> 166

<212> PRT

<213> Mycobacterium vaccae

<400> 109

Met Pro Val Arg Arg Ala Arg Ser Ala Leu Ala Ser Val Thr Phe Val

1	5	10	15
Ala Ala Ala Cys Val Gly Ala Glu Gly Thr Ala Leu Ala Ala Thr Pro			
20	25	30	
Asp Trp Ser Gly Arg Tyr Thr Val Val Thr Phe Ala Ser Asp Lys Leu			
35	40	45	
Gly Thr Ser Val Ala Ala Arg Gln Pro Glu Pro Asp Phe Ser Gly Gln			
50	55	60	
Tyr Thr Phe Ser Thr Ser Cys Val Gly Thr Cys Val Ala Thr Ala Ser			
65	70	75	80
Asp Gly Pro Ala Pro Ser Asn Pro Thr Ile Pro Gln Pro Ala Arg Tyr			
85	90	95	
Thr Trp Asp Gly Arg Gln Trp Val Phe Asn Tyr Asn Trp Gln Trp Glu			
100	105	110	
Cys Phe Arg Gly Ala Asp Val Pro Arg Glu Tyr Ala Ala Arg Ser			
115	120	125	
Leu Val Phe Tyr Ala Pro Thr Ala Asp Gly Ser Met Phe Gly Thr Trp			
130	135	140	
Arg Thr Asp Ile Leu Asp Gly Leu Cys Lys Gly Thr Val Ile Met Pro			
145	150	155	160
Val Ala Ala Tyr Pro Ala			
165			

<210> 110

<211> 74

<212> PRT

<213> Mycobacterium vaccae

<400> 110

Pro Arg Asp Thr His Pro Gly Ala Asn Gln Ala Val Thr Ala Ala Met			
1	5	10	15
Asn Gln Pro Arg Pro Glu Ala Glu Ala Asn Leu Arg Gly Tyr Phe Thr			
20	25	30	
Ala Asn Pro Ala Glu Tyr Tyr Asp Leu Arg Gly Ile Leu Ala Pro Ile			
35	40	45	
Gly Asp Ala Gln Arg Asn Cys Asn Ile Thr Val Leu Pro Val Glu Leu			
50	55	60	
Gln Thr Ala Tyr Asp Thr Phe Met Ala Gly			
65	70		

<210> 111

<211> 503

<212> DNA

<213> Mycobacterium vaccae

<220>

<221> unsure

<222> (358) ... (358)

<400> 111

atgcaggtgc	ggcgtgttct	gggcagtgtc	ggtgcagcag	tcgcggtttc	ggccgcgtta	60
tggcagacgg	gggtttcgat	accgaccgcc	tcagcggatc	cgtgtccgga	catcgaggtg	120
atcttcgcgc	gcgggaccgg	tgcggaaccc	ggcctcgggt	gggtcgggtga	tgcgttcgct	180
aacgcgctgc	ggcccaaggt	cggtgagcag	tcggtgggca	cctacgcggt	gaactaccgc	240

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gcaggattcg gacttcgaca aatcggcgcc catgggcgcg gccgacgcat cggggcgggg 300
gcagtggatg gccgacaact gcccgacac caagcttgct ctgggcgga tgctgcangg 360
cgccggcgctc atcgacctga tcaccgtcga tccgcgaccg ctgggcggt tccccccac 420
cccgatgccg ccccgcgctg ccgaccacgt ggccgcgctt gtggtcttcg gaaatccgtt 480
gcgcgacatc cgtggtggcg gtc 503

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<210> 112
 <211> 167
 <212> PRT
 <213> *Mycobacterium vaccae*

<220>
 <221> UNSURE
 <222> (119) ... (119)

<400> 112

Met	Gln	Val	Arg	Arg	Val	Leu	Gly	Ser	Val	Gly	Ala	Ala	Val	Ala	Val
1				5					10					15	
Ser	Ala	Ala	Leu	Trp	Gln	Thr	Gly	Val	Ser	Ile	Pro	Thr	Ala	Ser	Ala
			20					25					30		
Asp	Pro	Cys	Pro	Asp	Ile	Glu	Val	Ile	Phe	Ala	Arg	Gly	Thr	Gly	Ala
		35				40						45			
Glu	Pro	Gly	Leu	Gly	Trp	Val	Gly	Asp	Ala	Phe	Val	Asn	Ala	Leu	Arg
		50				55					60				
Pro	Lys	Val	Gly	Glu	Gln	Ser	Val	Gly	Thr	Tyr	Ala	Val	Asn	Tyr	Pro
65					70				75					80	
Ala	Gly	Phe	Asp	Phe	Asp	Lys	Ser	Ala	Pro	Met	Gly	Ala	Ala	Asp	Ala
			85					90					95		
Ser	Gly	Arg	Val	Gln	Trp	Met	Ala	Asp	Asn	Cys	Pro	Asp	Thr	Lys	Leu
		100						105					110		
Val	Leu	Gly	Gly	Met	Ser	Xaa	Gly	Ala	Gly	Val	Ile	Asp	Leu	Ile	Thr
		115					120					125			
Val	Asp	Pro	Arg	Pro	Leu	Gly	Arg	Phe	Thr	Pro	Thr	Pro	Met	Pro	Pro
	130					135					140				
Arg	Val	Ala	Asp	His	Val	Ala	Ala	Val	Val	Val	Phe	Gly	Asn	Pro	Leu
145					150					155					160
Arg	Asp	Ile	Arg	Gly	Gly	Gly									
				165											

<210> 113
 <211> 1569
 <212> DNA
 <213> *Mycobacterium vaccae*

<400> 113

atggccaaga	caattgcgta	tgacgaagag	gcccgcgctg	gcctcgagcg	gggcctcaac	60
gccctcgag	acgccgtaaa	ggtgacgttg	ggcccgaagg	gtcgcaacgt	cgtgctggag	120
aagaagtggg	gcgccccac	gatcaccaac	gatggtgtgt	ccatcgccaa	ggagatcgag	180
ctggaggacc	cgtacgagaa	gatcggcgct	gagctggtca	aagaggtcgc	caagaagacc	240
gacgacgtcg	cgggcgacgg	caccaccacc	gccaccgtgc	tcgctcaggc	tctggttcgc	300
gaaggcctgc	gcaacgtcgc	agccggcgcc	aaccgcctcg	gcctcaagcg	tggcatcgag	360
aaggctgtcg	aggctgtcac	ccagtcgctg	ctgaagtcgg	ccaaggaggt	cgagaccaag	420
gagcagattt	ctgccaccgc	ggcgatttcc	gccggcgaca	cccagatcgg	cgagctcatc	480


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gccgaggcca tggacaaggt cggcaacgag ggtgtcatca ccgtcgagga gtcgaacacc      540
ttcggcctgc agctcgagct caccgagggg atgcgcttcg acaagggcta catctcgggt      600
tacttcgtga ccgacgccga gcgccaggaa gccgtcctgg aggatcccta catcctgctg      660
gtcagctcca aggtgtcgac cgtcaaggat ctgctcccgc tgctggagaa ggtcatccag      720
gccggcaagc cgctgctgat catcgccgag gacgtcgagg gcgaggccct gtccacgctg      780
gtgggtcaaca agatccgcgg caccttcaag tccgtcgccg tcaaggctcc gggcttcggg      840
gaccgccgca aggcgatgct gcaggacatg gccatcctca ccggtgggtca ggtcgtcagc      900
gaaagagtcg ggctgtccct ggagaccgcc gacgtctcgc tgctgggcca ggcccgaag      960
gtcgtcgtca ccaaggacga gaccaccatc gtcgagggtc cgggcgattc cgatgccatc     1020
gccggccggg tggctcagat ccgcgccgag atcgagaaca gcgactccga ctacgaccgc     1080
gagaagctgc aggagcgctt ggccaagctg gccggcggtg ttgcggtgat caaggccgga     1140
gctgccaccg aggtggagct caaggagcgc aagcaccgca tcgaggacgc cgtccgcaac     1200
gcgaaggctg ccgtcgaaga gggcatcgct gccggtggcg gcgtggctct gctgcagtcg     1260
gctcctgcgc tggacgacct cggcctgacg ggcgacgagg ccaccggtgc caacatcgctc     1320
cgctgtggcg tgctggctcc gctcaagcag atcgcttca acggcgccct ggagcccggc     1380
gtcgttgccg agaaggtgtc caacctgccc gcgggtcacg gcctcaacgc cgcgaccggt     1440
gagtacgagg acctgctcaa ggccggcgct gccgaccggt tgaaggtcac ccgctcggcg     1500
ctgcagaacg cggcgctccat cgcggctctg ttcctcacca ccgaggccgt cgtcgccgac     1560
aagccggag                                     1569

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<210> 114

<211> 523

<212> PRT

<213> Mycobacterium vaccae

<400> 114

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Met Ala Lys Thr Ile Ala Tyr Asp Glu Glu Ala Arg Arg Gly Leu Glu
 1             5             10             15
Arg Gly Leu Asn Ala Leu Ala Asp Ala Val Lys Val Thr Leu Gly Pro
      20             25             30
Lys Gly Arg Asn Val Val Leu Glu Lys Lys Trp Gly Ala Pro Thr Ile
      35             40             45
Thr Asn Asp Gly Val Ser Ile Ala Lys Glu Ile Glu Leu Glu Asp Pro
      50             55             60
Tyr Glu Lys Ile Gly Ala Glu Leu Val Lys Glu Val Ala Lys Lys Thr
      65             70             75             80
Asp Asp Val Ala Gly Asp Gly Thr Thr Thr Ala Thr Val Leu Ala Gln
      85             90             95
Ala Leu Val Arg Glu Gly Leu Arg Asn Val Ala Ala Gly Ala Asn Pro
      100            105            110
Leu Gly Leu Lys Arg Gly Ile Glu Lys Ala Val Glu Ala Val Thr Gln
      115            120            125
Ser Leu Leu Lys Ser Ala Lys Glu Val Glu Thr Lys Glu Gln Ile Ser
      130            135            140
Ala Thr Ala Ala Ile Ser Ala Gly Asp Thr Gln Ile Gly Glu Leu Ile
      145            150            155            160
Ala Glu Ala Met Asp Lys Val Gly Asn Glu Gly Val Ile Thr Val Glu
      165            170            175
Glu Ser Asn Thr Phe Gly Leu Gln Leu Glu Leu Thr Glu Gly Met Arg
      180            185            190
Phe Asp Lys Gly Tyr Ile Ser Gly Tyr Phe Val Thr Asp Ala Glu Arg
      195            200            205
Gln Glu Ala Val Leu Glu Asp Pro Tyr Ile Leu Leu Val Ser Ser Lys

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210                215                220
Val Ser Thr Val Lys Asp Leu Leu Pro Leu Leu Glu Lys Val Ile Gln
225                230                235                240
Ala Gly Lys Pro Leu Leu Ile Ile Ala Glu Asp Val Glu Gly Glu Ala
245                250                255
Leu Ser Thr Leu Val Val Asn Lys Ile Arg Gly Thr Phe Lys Ser Val
260                265                270
Ala Val Lys Ala Pro Gly Phe Gly Asp Arg Arg Lys Ala Met Leu Gln
275                280                285
Asp Met Ala Ile Leu Thr Gly Gly Gln Val Val Ser Glu Arg Val Gly
290                295                300
Leu Ser Leu Glu Thr Ala Asp Val Ser Leu Leu Gly Gln Ala Arg Lys
305                310                315                320
Val Val Val Thr Lys Asp Glu Thr Thr Ile Val Glu Gly Ser Gly Asp
325                330                335
Ser Asp Ala Ile Ala Gly Arg Val Ala Gln Ile Arg Ala Glu Ile Glu
340                345                350
Asn Ser Asp Ser Asp Tyr Asp Arg Glu Lys Leu Gln Glu Arg Leu Ala
355                360                365
Lys Leu Ala Gly Gly Val Ala Val Ile Lys Ala Gly Ala Ala Thr Glu
370                375                380
Val Glu Leu Lys Glu Arg Lys His Arg Ile Glu Asp Ala Val Arg Asn
385                390                395                400
Ala Lys Ala Ala Val Glu Glu Gly Ile Val Ala Gly Gly Gly Val Ala
405                410                415
Leu Leu Gln Ser Ala Pro Ala Leu Asp Asp Leu Gly Leu Thr Gly Asp
420                425                430
Glu Ala Thr Gly Ala Asn Ile Val Arg Val Ala Leu Ser Ala Pro Leu
435                440                445
Lys Gln Ile Ala Phe Asn Gly Gly Leu Glu Pro Gly Val Val Ala Glu
450                455                460
Lys Val Ser Asn Leu Pro Ala Gly His Gly Leu Asn Ala Ala Thr Gly
465                470                475                480
Glu Tyr Glu Asp Leu Leu Lys Ala Gly Val Ala Asp Pro Val Lys Val
485                490                495
Thr Arg Ser Ala Leu Gln Asn Ala Ala Ser Ile Ala Ala Leu Phe Leu
500                505                510
Thr Thr Glu Ala Val Val Ala Asp Lys Pro Glu
515                520

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<210> 115

<211> 647

<212> DNA

<213> Mycobacterium vaccae

<400> 115

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atggccaaga caattgcgta tgacgaagag gcccgccgtg gcctcgagcg gggcctcaac      60
gccctcgag  acgccgtaaa ggtgacgttg ggcccgaagg gtcgcaacgt cgtgctggag      120
aagaagtggg gcgccccac  gatcaccaac gatggtgtgt ccatcgccaa ggagatcgag      180
ctggaggacc cgtaacgagaa  gatcggcgct gagctggtca aagaggtcgc caagaagacc      240
gacgacgtcg cgggagacgg  caccaccacc gccaccgtgc tcgctcagge tctggttcgc      300
gaaggcctgc gcaacgtcgc  agccggcgcc aaccgcgtcg gcctcaagcg tggcatcgag      360
aaggctgtcg aggtgtcac  ccagtcgctg ctgaagtcgg ccaaggaggt cgagaccaag      420

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gagcagattt	ctgccaccgc	ggcgatttcc	gccggcgaca	cccagatcgg	cgagctcatc	480
gccgaggcca	tggacaaggt	cggcaacgag	ggtgtcatca	ccgtcgagga	gtcgaacacc	540
ttcggcctgc	agctcgagct	caccgagggt	atgcgcttcg	acaagggcta	catctcgggt	600
tacttcgtga	ccgacgccga	gcgccaggaa	gccgtcctgg	aggatcc		647

<210> 116

<211> 927

<212> DNA

<213> Mycobacterium vaccae

<400> 116

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gaggccctgt	ccacgtctggt	ggtcaacaag	atccgcggca	ccttcaagtc	cgtcgccgtc	180
aaggctccgg	gcttcggtga	ccgccgcaag	gcgatgctgc	aggacatggc	catcctcacc	240
ggtggtcagg	tcgtcagcga	aagagtcggg	ctgtccctgg	agaccgccga	cgtctcgctg	300
ctgggccagg	cccgaaggt	cgctcgtcacc	aaggacgaga	ccaccatcgt	cgagggctcg	360
ggcgattccg	atgccatcgc	cggccgggtg	gtcagatcc	gcgccgagat	cgagaacagc	420
gactccgact	acgaccgcga	gaagctgcag	gagcgcttgg	ccaagctggc	cggcggtgtt	480
gcggtgatca	aggccggagc	tgccaccgag	gtggagctca	aggagcgcaa	gcaccgcata	540
gaggacgccg	tccgcaacgc	gaaggctgcc	gtcgaagagg	gcatacgtcgc	cgggtggcggc	600
gtggctctgc	tgcagtccgc	tctgctgctg	gacgacctcg	gcctgacggg	cgacgagggc	660
accggtgcc	acatcgctccg	cgtggcgctg	tcggctccgc	tcaagcagat	cgcttcaac	720
ggcggcctgg	agcccggtg	cgttgccgag	aagggtgtcca	acctgcccgc	gggtcacggc	780
ctcaacgccg	cgaccggtga	gtaccgaggac	ctgctcaagg	ccggcgctgc	cgacccggtg	840
aaggtcaccc	gctcggtcgt	gcagaacgcg	gcgtccatcg	cggtctgtgt	cctcaccacc	900
gaggccgtcg	tcgccgacaa	gccggag				927

<210> 117

<211> 215

<212> PRT

<213> Mycobacterium vaccae

<400> 117

Met	Ala	Lys	Thr	Ile	Ala	Tyr	Asp	Glu	Glu	Ala	Arg	Arg	Gly	Leu	Glu
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Arg	Gly	Leu	Asn	Ala	Leu	Ala	Asp	Ala	Val	Lys	Val	Thr	Leu	Gly	Pro
		20					25						30		
Lys	Gly	Arg	Asn	Val	Val	Leu	Glu	Lys	Lys	Trp	Gly	Ala	Pro	Thr	Ile
		35				40					45				
Thr	Asn	Asp	Gly	Val	Ser	Ile	Ala	Lys	Glu	Ile	Glu	Leu	Glu	Asp	Pro
	50				55				60						
Tyr	Glu	Lys	Ile	Gly	Ala	Glu	Leu	Val	Lys	Glu	Val	Ala	Lys	Lys	Thr
	65			70				75						80	
Asp	Asp	Val	Ala	Gly	Asp	Gly	Thr	Thr	Thr	Ala	Thr	Val	Leu	Ala	Gln
			85				90						95		
Ala	Leu	Val	Arg	Glu	Gly	Leu	Arg	Asn	Val	Ala	Ala	Gly	Ala	Asn	Pro
		100					105					110			
Leu	Gly	Leu	Lys	Arg	Gly	Ile	Glu	Lys	Ala	Val	Glu	Ala	Val	Thr	Gln
	115					120					125				
Ser	Leu	Leu	Lys	Ser	Ala	Lys	Glu	Val	Glu	Thr	Lys	Glu	Gln	Ile	Ser
	130					135					140				
Ala	Thr	Ala	Ala	Ile	Ser	Ala	Gly	Asp	Thr	Gln	Ile	Gly	Glu	Leu	Ile

145		150		155		160									
Ala	Glu	Ala	Met	Asp	Lys	Val	Gly	Asn	Glu	Gly	Val	Ile	Thr	Val	Glu
			165					170						175	
Glu	Ser	Asn	Thr	Phe	Gly	Leu	Gln	Leu	Glu	Leu	Thr	Glu	Gly	Met	Arg
		180					185						190		
Phe	Asp	Lys	Gly	Tyr	Ile	Ser	Gly	Tyr	Phe	Val	Thr	Asp	Ala	Glu	Arg
	195					200						205			
Gln	Glu	Ala	Val	Leu	Glu	Asp									
	210					215									

<210> 118
 <211> 309
 <212> PRT
 <213> Mycobacterium vaccae

<400> 118

Asp	Pro	Tyr	Ile	Leu	Leu	Val	Ser	Ser	Lys	Val	Ser	Thr	Val	Lys	Asp
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Leu	Leu	Pro	Leu	Leu	Glu	Lys	Val	Ile	Gln	Ala	Gly	Lys	Pro	Leu	Leu
		20					25						30		
Ile	Ile	Ala	Glu	Asp	Val	Glu	Gly	Glu	Ala	Leu	Ser	Thr	Leu	Val	Val
	35					40						45			
Asn	Lys	Ile	Arg	Gly	Thr	Phe	Lys	Ser	Val	Ala	Val	Lys	Ala	Pro	Gly
	50				55						60				
Phe	Gly	Asp	Arg	Arg	Lys	Ala	Met	Leu	Gln	Asp	Met	Ala	Ile	Leu	Thr
65				70					75					80	
Gly	Gly	Gln	Val	Val	Ser	Glu	Arg	Val	Gly	Leu	Ser	Leu	Glu	Thr	Ala
			85						90					95	
Asp	Val	Ser	Leu	Leu	Gly	Gln	Ala	Arg	Lys	Val	Val	Val	Thr	Lys	Asp
		100					105						110		
Glu	Thr	Thr	Ile	Val	Glu	Gly	Ser	Gly	Asp	Ser	Asp	Ala	Ile	Ala	Gly
	115					120						125			
Arg	Val	Ala	Gln	Ile	Arg	Ala	Glu	Ile	Glu	Asn	Ser	Asp	Ser	Asp	Tyr
	130				135						140				
Asp	Arg	Glu	Lys	Leu	Gln	Glu	Arg	Leu	Ala	Lys	Leu	Ala	Gly	Gly	Val
145				150					155					160	
Ala	Val	Ile	Lys	Ala	Gly	Ala	Ala	Thr	Glu	Val	Glu	Leu	Lys	Glu	Arg
			165					170						175	
Lys	His	Arg	Ile	Glu	Asp	Ala	Val	Arg	Asn	Ala	Lys	Ala	Ala	Val	Glu
		180					185						190		
Glu	Gly	Ile	Val	Ala	Gly	Gly	Gly	Val	Ala	Leu	Leu	Gln	Ser	Ala	Pro
	195					200						205			
Ala	Leu	Asp	Asp	Leu	Gly	Leu	Thr	Gly	Asp	Glu	Ala	Thr	Gly	Ala	Asn
	210					215						220			
Ile	Val	Arg	Val	Ala	Leu	Ser	Ala	Pro	Leu	Lys	Gln	Ile	Ala	Phe	Asn
225				230						235					240
Gly	Gly	Leu	Glu	Pro	Gly	Val	Val	Ala	Glu	Lys	Val	Ser	Asn	Leu	Pro
				245					250					255	
Ala	Gly	His	Gly	Leu	Asn	Ala	Ala	Thr	Gly	Glu	Tyr	Glu	Asp	Leu	Leu
		260					265						270		
Lys	Ala	Gly	Val	Ala	Asp	Pro	Val	Lys	Val	Thr	Arg	Ser	Ala	Leu	Gln
	275					280						285			
Asn	Ala	Ala	Ser	Ile	Ala	Ala	Leu	Phe	Leu	Thr	Thr	Glu	Ala	Val	Val

290
Ala Asp Lys Pro Glu
305

295

300

<210> 119
<211> 162
<212> DNA
<213> Mycobacterium vaccae

<400> 119
ctcgtacagg cgacggagat ctccgacgac gccacgtcgg tacggttggt cgccaccctg 60
ttcggcgctcg tgttggtgac gttggtgctg tccgggctca acgccaccct catccagggc 120
gcaccagaag acagctggcg caggcgggatt ccgtcgatct tc 162

<210> 120
<211> 1366
<212> DNA
<213> Mycobacterium vaccae

<220>
<221> unsure
<222> (955) ... (955)

<221> unsure
<222> (973) ... (973)

<400> 120
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cccgggtgctg ctgggtcgctg tgaccgaggt gcacaacgcg ttgcgtcggc gcggcagcgc 120
gctggcccg cgggtgcaac tcctgctgac ctacatcctg ccgctgggcg cggttgcgtgct 180
cctgctggta caggcgatgg agatctccga cgacgccacg tcggtagcgt tggtcgccac 240
cctgttcggc gtgctgttgt tgacgttggt gctgtccggg ctcaacgcca ccctcatcca 300
gggcgcacca gaagacagct ggcgcaggcg gattccgtcg atcttccctg acgtcgcgcg 360
cttcgcgctg atcgcggctg gtatcacctg gatcatggcc tatgtctggg gcgcgaacgt 420
ggggggcctg ttcaccgcac tgggcgtcac ttccatcgtt cttggcctgg ctctgcagaa 480
ttcggtcggt cagatcatct cgggtctgct gctgctgttc gagcaaccgt tccggctcgg 540
cgactggatc accgtcccca ccgcggcggg ccggccgtcc gccacggcc gcgtggtgga 600
agtcaactgg cgtgcaacac atatcgacac cggcggcaac ctgctggtaa tgccaacgc 660
cgaactcgcc ggcgcgctcg tcaccaatta cagccggccc gtgggagagc accggctgac 720
cgctcgtcacc accttcaacg ccgcggacac ccccgatgat gtctgcgaga tgctgtcgtc 780
ggtcgcggcg tcgctgcccg aactgcgcac cgacggacag atcgccacgc tctatctcgg 840
tgcgcccgaa tacgagaagt cgatcccggt gcacacaccc gcggtggacg actcggtcag 900
gagcacgtac ctgcgatggg tctggtacgc cgcgcgccgg caggaaacttc gcctnaacgg 960
cgtcgccgac ganttcgaca cgccggaacg gatcgccctg gccatgcggg ctgtggcgctc 1020
cacactgcgc ttggcagacg acgaacagca ggagatcgcc gacgtggtgc gtctggtccg 1080
ttacggcaac ggggaacgcc tccagcagcc gggtcaggta ccgaccggga tgaggttcat 1140
cgtagacggc agggtagtc tgccgtgat cgatcaggac ggcgacgtga tcccggcgcg 1200
ggtgctcgag cgtggcgact tcctggggca gaccacgctg acgcgggaac cggtagtggc 1260
gaccgcgcac gcgctggagg aagtcaccgt gctggagatg gcccgtagc agatcgagcg 1320
cctggtgcac cgaaagccga tcctgctgca cgtgatcggg gccgtg 1366

<210> 121
<211> 455

<212> PRT

<213> Mycobacterium vaccae

<220>

<221> UNSURE

<222> (318) ... (318)

<221> UNSURE

<222> (324) ... (324)

<400> 121

Met	Ser	Ser	Val	Leu	Asn	Ser	Thr	Trp	Leu	Ala	Trp	Ala	Val	Ala	Val
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Ala	Val	Gly	Phe	Pro	Val	Leu	Leu	Val	Val	Leu	Thr	Glu	Val	His	Asn
		20						25					30		
Ala	Leu	Arg	Arg	Arg	Gly	Ser	Ala	Leu	Ala	Arg	Pro	Val	Gln	Leu	Leu
		35					40					45			
Arg	Thr	Tyr	Ile	Leu	Pro	Leu	Gly	Ala	Leu	Leu	Leu	Leu	Leu	Val	Gln
	50					55					60				
Ala	Met	Glu	Ile	Ser	Asp	Asp	Ala	Thr	Ser	Val	Arg	Leu	Val	Ala	Thr
65					70					75					80
Leu	Phe	Gly	Val	Val	Leu	Leu	Thr	Leu	Val	Leu	Ser	Gly	Leu	Asn	Ala
				85					90					95	
Thr	Leu	Ile	Gln	Gly	Ala	Pro	Glu	Asp	Ser	Trp	Arg	Arg	Arg	Ile	Pro
			100					105					110		
Ser	Ile	Phe	Leu	Asp	Val	Ala	Arg	Phe	Ala	Leu	Ile	Ala	Val	Gly	Ile
		115					120					125			
Thr	Val	Ile	Met	Ala	Tyr	Val	Trp	Gly	Ala	Asn	Val	Gly	Gly	Leu	Phe
	130					135					140				
Thr	Ala	Leu	Gly	Val	Thr	Ser	Ile	Val	Leu	Gly	Leu	Ala	Leu	Gln	Asn
145					150					155					160
Ser	Val	Gly	Gln	Ile	Ile	Ser	Gly	Leu	Leu	Leu	Leu	Phe	Glu	Gln	Pro
			165						170					175	
Phe	Arg	Leu	Gly	Asp	Trp	Ile	Thr	Val	Pro	Thr	Ala	Ala	Gly	Arg	Pro
		180						185					190		
Ser	Ala	His	Gly	Arg	Val	Val	Glu	Val	Asn	Trp	Arg	Ala	Thr	His	Ile
		195					200					205			
Asp	Thr	Gly	Gly	Asn	Leu	Leu	Val	Met	Pro	Asn	Ala	Glu	Leu	Ala	Gly
	210					215					220				
Ala	Ser	Phe	Thr	Asn	Tyr	Ser	Arg	Pro	Val	Gly	Glu	His	Arg	Leu	Thr
225					230					235					240
Val	Val	Thr	Thr	Phe	Asn	Ala	Ala	Asp	Thr	Pro	Asp	Asp	Val	Cys	Glu
				245					250					255	
Met	Leu	Ser	Ser	Val	Ala	Ala	Ser	Leu	Pro	Glu	Leu	Arg	Thr	Asp	Gly
			260					265					270		
Gln	Ile	Ala	Thr	Leu	Tyr	Leu	Gly	Ala	Ala	Glu	Tyr	Glu	Lys	Ser	Ile
		275					280					285			
Pro	Leu	His	Thr	Pro	Ala	Val	Asp	Asp	Ser	Val	Arg	Ser	Thr	Tyr	Leu
	290					295					300				
Arg	Trp	Val	Trp	Tyr	Ala	Ala	Arg	Arg	Gln	Glu	Leu	Arg	Xaa	Asn	Gly
305					310					315					320
Val	Ala	Asp	Xaa	Phe	Asp	Thr	Pro	Glu	Arg	Ile	Ala	Ser	Ala	Met	Arg
				325					330					335	

Ala Val Ala Ser Thr Leu Arg Leu Ala Asp Asp Glu Gln Gln Glu Ile
 340 345 350
 Ala Asp Val Val Arg Leu Val Arg Tyr Gly Asn Gly Glu Arg Leu Gln
 355 360 365
 Gln Pro Gly Gln Val Pro Thr Gly Met Arg Phe Ile Val Asp Gly Arg
 370 375 380
 Val Ser Leu Ser Val Ile Asp Gln Asp Gly Asp Val Ile Pro Ala Arg
 385 390 395 400
 Val Leu Glu Arg Gly Asp Phe Leu Gly Gln Thr Thr Leu Thr Arg Glu
 405 410 415
 Pro Val Leu Ala Thr Ala His Ala Leu Glu Glu Val Thr Val Leu Glu
 420 425 430
 Met Ala Arg Asp Glu Ile Glu Arg Leu Val His Arg Lys Pro Ile Leu
 435 440 445
 Leu His Val Ile Gly Ala Val
 450 455

<210> 122

<211> 898

<212> DNA

<213> Mycobacterium vaccae

<400> 122

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cgctaccacc	tctgtcgcg	gatgagcatc	cagtccaagt	tgctgctgat	gctgcttctg	120
accagcattc	tctcggctgc	ggtggtcggt	ttcatcggct	atcagtcggt	acggctctcg	180
ctgcgcgcac	cgggtgttga	ccgcctcacc	gacatccggt	agtcgcagtc	gcgcggggtg	240
gagaatcagt	tcgcggacct	gaagaactcg	atggtgattt	actcgcgcgg	cagcactgcc	300
acggaggcga	tcggcgcgtt	cagcgacggt	ttccgtcagc	tcggcgcgat	gacgatcaat	360
accgggaggg	cggcgtcatt	gcgcgcgttac	tacgaccgga	cgcttcgcaa	caccaccctc	420
gacgacagcg	gaaaccgcgt	cgacgtccgc	gcgctcatcc	cgaaatccaa	ccccagcgcc	480
tatctgcagg	cgctctatac	cccgcggttt	cagaactggg	agaaggcgat	cgcggttcgac	540
gacgcgcgcg	acggcagcgc	ctggtcggcc	gccaatgcca	gattcaacga	gttcttccgc	600
gagatcgtgc	accgcttcaa	cttcgaggat	ctgatgctgc	tcgacctcga	gggcaacgtg	660
gtgtactccg	cctacaaggg	gccggatctc	gggacaaaca	tcgtcaacgg	cccctatcgc	720
aaccgggaac	tgctcggaagc	ctacgagaag	gcggtcgcgt	cgaactcgat	cgactatgtc	780
ggtgtcaccg	acttcgggtg	gtacctgcct	gccgaggaaac	cgaccgcctg	gttctctgtc	840
ccggtcgggt	tgaaggaccg	agtcgacggt	gtgatggcgg	tccagttccc	cggaattc	898

<210> 123

<211> 1259

<212> DNA

<213> Mycobacterium vaccae

<400> 123

cgcaattgat	gacggcgcgg	ggacagtggc	gtgacaccgg	gatgggagac	accggtgaga	60
ccatcctggt	cggaccggac	aatctgatgc	gctcggactc	ccggctgttc	cgcgagaacc	120
gggagaagtt	cctggccgac	gtcgtcgagg	ggggaaaccc	gccggagggt	gccgacgaat	180
cggttgaccg	ccgcggcacc	acgctggtgc	agccggtgac	caccgcctcc	gtcgaggagg	240
cccaacgcgg	caacaccggg	acgacgateg	aggacgacta	tctcggccac	gaggcggttac	300
aggcgtactc	accggtggac	ctgccgggac	tgactgggtg	gatcgtggcc	aagatcgaca	360
ccgacgaggg	gttcgccccg	gtggcgcagt	tcaccaggac	cctggtgctg	tcgacgggtga	420
tcatcatctt	cggcgtgtcg	ctggcggcca	tgctgctggc	gcggttggtc	gtccgtccga	480

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tccggcggtt gcaggccggc gccagcaga tcagcggcgg tgactaccgc ctgctctgc 540
cgggtgttgtc tcgtgacgaa ttcggcgatc tgacaacagc tttcaacgac atgagtcgca 600
atctgtcgat caaggacgag ctgctcggcg aggagcgcgc cgagaaccaa cggctgatgc 660
tgtccctgat gccgaaccg gtgatgcagc gctacctcga cggggaggag acgatcgccc 720
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gcatgttgac ctccgaggaa ctgatggtgg tggcacaaga cctgaccgcg cagttcgacg 840
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aaatggaccg catcatcgac cggcacgccg ccgagtcggg gcacgacctg cggctccgcg 1020
cgggcatcga caccgggtcg gcggccagcg ggctgggtgg gcggtccacg ttggcgtacg 1080
acatgtgggg ttcggcggtc gatgtcgct accaggtgca gcggtccacg cccagccccg 1140
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<210> 124

<211> 299

<212> PRT

<213> Mycobacterium vaccae

<400> 124

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Met Thr Ile Leu Pro Trp Asn Ala Arg Thr Ser Glu His Pro Thr Arg
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20 25 30
Lys Leu Leu Leu Met Leu Leu Leu Thr Ser Ile Leu Ser Ala Ala Val
35 40 45
Val Gly Phe Ile Gly Tyr Gln Ser Gly Arg Ser Ser Leu Arg Ala Ser
50 55 60
Val Phe Asp Arg Leu Thr Asp Ile Arg Glu Ser Gln Ser Arg Gly Leu
65 70 75 80
Glu Asn Gln Phe Ala Asp Leu Lys Asn Ser Met Val Ile Tyr Ser Arg
85 90 95
Gly Ser Thr Ala Thr Glu Ala Ile Gly Ala Phe Ser Asp Gly Phe Arg
100 105 110
Gln Leu Gly Asp Ala Thr Ile Asn Thr Gly Gln Ala Ala Ser Leu Arg
115 120 125
Arg Tyr Tyr Asp Arg Thr Phe Ala Asn Thr Thr Leu Asp Asp Ser Gly
130 135 140
Asn Arg Val Asp Val Arg Ala Leu Ile Pro Lys Ser Asn Pro Gln Arg
145 150 155 160
Tyr Leu Gln Ala Leu Tyr Thr Pro Pro Phe Gln Asn Trp Glu Lys Ala
165 170 175
Ile Ala Phe Asp Asp Ala Arg Asp Gly Ser Ala Trp Ser Ala Ala Asn
180 185 190
Ala Arg Phe Asn Glu Phe Phe Arg Glu Ile Val His Arg Phe Asn Phe
195 200 205
Glu Asp Leu Met Leu Leu Asp Leu Glu Gly Asn Val Val Tyr Ser Ala
210 215 220
Tyr Lys Gly Pro Asp Leu Gly Thr Asn Ile Val Asn Gly Pro Tyr Arg
225 230 235 240
Asn Arg Glu Leu Ser Glu Ala Tyr Glu Lys Ala Val Ala Ser Asn Ser
245 250 255
Ile Asp Tyr Val Gly Val Thr Asp Phe Gly Trp Tyr Leu Pro Ala Glu

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260 265 270
 Glu Pro Thr Ala Trp Phe Leu Ser Pro Val Gly Leu Lys Asp Arg Val
 275 280 285
 Asp Gly Val Met Ala Val Gln Phe Pro Gly Ile
 290 295

<210> 125
 <211> 419
 <212> PRT
 <213> Mycobacterium vaccae

<400> 125
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 20 25 30
 Ser Arg Leu Phe Arg Glu Asn Arg Glu Lys Phe Leu Ala Asp Val Val
 35 40 45
 Glu Gly Gly Thr Pro Pro Glu Val Ala Asp Glu Ser Val Asp Arg Arg
 50 55 60
 Gly Thr Thr Leu Val Gln Pro Val Thr Thr Arg Ser Val Glu Glu Ala
 65 70 75 80
 Gln Arg Gly Asn Thr Gly Thr Thr Ile Glu Asp Asp Tyr Leu Gly His
 85 90 95
 Glu Ala Leu Gln Ala Tyr Ser Pro Val Asp Leu Pro Gly Leu His Trp
 100 105 110
 Val Ile Val Ala Lys Ile Asp Thr Asp Glu Ala Phe Ala Pro Val Ala
 115 120 125
 Gln Phe Thr Arg Thr Leu Val Leu Ser Thr Val Ile Ile Ile Phe Gly
 130 135 140
 Val Ser Leu Ala Ala Met Leu Leu Ala Arg Leu Phe Val Arg Pro Ile
 145 150 155 160
 Arg Arg Leu Gln Ala Gly Ala Gln Gln Ile Ser Gly Gly Asp Tyr Arg
 165 170 175
 Leu Ala Leu Pro Val Leu Ser Arg Asp Glu Phe Gly Asp Leu Thr Thr
 180 185 190
 Ala Phe Asn Asp Met Ser Arg Asn Leu Ser Ile Lys Asp Glu Leu Leu
 195 200 205
 Gly Glu Glu Arg Ala Glu Asn Gln Arg Leu Met Leu Ser Leu Met Pro
 210 215 220
 Glu Pro Val Met Gln Arg Tyr Leu Asp Gly Glu Glu Thr Ile Ala Gln
 225 230 235 240
 Asp His Lys Asn Val Thr Val Ile Phe Ala Asp Met Met Gly Leu Asp
 245 250 255
 Glu Leu Ser Arg Met Leu Thr Ser Glu Glu Leu Met Val Val Val Asn
 260 265 270
 Asp Leu Thr Arg Gln Phe Asp Ala Ala Ala Glu Ser Leu Gly Val Asp
 275 280 285
 His Val Arg Thr Leu His Asp Gly Tyr Leu Ala Ser Cys Gly Leu Gly
 290 295 300
 Val Pro Arg Leu Asp Asn Val Arg Arg Thr Val Asn Phe Ala Ile Glu
 305 310 315 320
 Met Asp Arg Ile Ile Asp Arg His Ala Ala Glu Ser Gly His Asp Leu

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<220>
<223> Made in a lab
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27

<220>
<223> Made in a lab

26

<220>
<223> Made in a lab

33

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<223> Made in a lab

<400> 129

ccggatccat tcggtggccc tgcaaccgcc ag

32

<210> 130

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Made in a lab

<400> 130

ccggatccgg agcaaccgtt ccggctc

27

<210> 131

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Made in a lab

<400> 131

ccggatcccg gctatcagtc cggacgg

27

<210> 132

<211> 844

<212> DNA

<213> Mycobacterium vaccae

<400> 132

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gcccacggcc	gcgtgggtgga	agtcaactgg	cgtgcaacac	atatcgacac	ccggcggcaac	120
ctgctggttaa	tgcccaacgc	cgaactcgcc	ggcgcgtcgt	tcaccaatta	cagccggccc	180
gtgggagagc	accggctgac	cgctcgtcacc	accttcaacg	ccgcggacac	ccccgatgat	240
gtctgcgaga	tgctgtcgtc	ggctcgcggcg	tcgctgcccg	aactgcgcac	cgacggacag	300
atcgccacgc	tctatctcgg	tgcgcccgaa	tacgagaagt	cgatcccgtt	gcacacaccc	360
gcggtggacg	actcggtcag	gagcacgtac	ctgcgatggg	tctggtacgc	cgcgcgccgg	420
caggaacttc	gcctaacggc	gtcgccgacg	attcgacacg	ccggaacgga	tcgcctcggc	480
catgcgggct	gtggcgtcca	cactgcgctt	ggcagacgac	gaacagcagg	agatcgccga	540
cgtggtgctg	ctgggtccgtt	acggcaacgg	ggaacgcctc	cagcagccgg	gtcaggtacc	600
gaccgggatg	aggttcatcg	tagacggcag	ggtgagtctg	tccgtgatcg	atcaggacgg	660
cgacgtgatc	ccggcgccgg	tgctcgagcg	tggcgacttc	ctggggcaga	ccacgctgac	720
gcgggaaccg	gtactggcga	ccgcgcacgc	gctggaggaa	gtcaccgtgc	tggagatggc	780
ccgtgacgag	atcgagcgcc	tggtgcaccg	aaagccgatc	ctgctgcacg	tgatcggggc	840
cgtg						844

<210> 133

<211> 742

<212> DNA

<213> Mycobacterium vaccae

<400> 133
 ggctatcagt ccggaacggc ctcgctgcgc gcatcggtgt tcgaccgcct caccgacatc 60
 cgcgagtcgc agtcgcgcgc gttggagaat cagttcgcgc acctgaagaa ctcgatgggtg 120
 atttactcgc gcggcagcac tgccacggag gcgatcggcg cgttcagcga cggtttccgt 180
 cagctcggcg atgcgacgat caataccggg caggcggcgt cattgcgcgc ttactacgac 240
 cggacgttcg ccaacaccac cctcgacgac agcggaaacc gcgtcgacgt ccgcgcgctc 300
 atcccgaat ccaacccccca gcgctatctg caggcgtctt ataccccgcc gtttcagaac 360
 tgggagaagg cgatcgcggt cgacgacgcg cgcgacggca gcgcctggtc ggccgccaat 420
 gccagattca acgagttctt ccgcgagatc gtgcaccgct tcaacttcga ggatctgatg 480
 ctgctcgacc tcgagggcaa cgtggtgtac tccgcctaca aggggcccga tctcgggaca 540
 aacatcgtca acggccccta tcgcaaccgg gaactgtcgg aagcctacga gaaggcggtc 600
 gcgtcgaact cgatcgacta tgcggtgtc accgacttcg ggtggtacct gcctgccgag 660
 gaaccgaccg cctggttcct gtccccggtc ggggtgaagg accgagtcga cgggtgtgatg 720
 gcggtccagt tccccggaat tc 742

<210> 134
 <211> 282
 <212> PRT
 <213> Mycobacterium vaccae

<220>
 <221> UNSURE
 <222> (145)... (145)

<221> UNSURE
 <222> (151)... (151)

<400> 134
 Glu Gln Pro Phe Arg Leu Gly Asp Trp Ile Thr Val Pro Thr Ala Ala
 1 5 10 15
 Gly Arg Pro Ser Ala His Gly Arg Val Val Glu Val Asn Trp Arg Ala
 20 25 30
 Thr His Ile Asp Thr Gly Gly Asn Leu Leu Val Met Pro Asn Ala Glu
 35 40 45
 Leu Ala Gly Ala Ser Phe Thr Asn Tyr Ser Arg Pro Val Gly Glu His
 50 55 60
 Arg Leu Thr Val Val Thr Thr Phe Asn Ala Ala Asp Thr Pro Asp Asp
 65 70 75 80
 Val Cys Glu Met Leu Ser Ser Val Ala Ala Ser Leu Pro Glu Leu Arg
 85 90 95
 Thr Asp Gly Gln Ile Ala Thr Leu Tyr Leu Gly Ala Ala Glu Tyr Glu
 100 105 110
 Lys Ser Ile Pro Leu His Thr Pro Ala Val Asp Asp Ser Val Arg Ser
 115 120 125
 Thr Tyr Leu Arg Trp Val Trp Tyr Ala Ala Arg Arg Gln Glu Leu Arg
 130 135 140
 Xaa Asn Gly Val Ala Asp Xaa Phe Asp Thr Pro Glu Arg Ile Ala Ser
 145 150 155 160
 Ala Met Arg Ala Val Ala Ser Thr Leu Arg Leu Ala Asp Asp Glu Gln
 165 170 175
 Gln Glu Ile Ala Asp Val Val Arg Leu Val Arg Tyr Gly Asn Gly Glu
 180 185 190

Arg Leu Gln Gln Pro Gly Gln Val Pro Thr Gly Met Arg Phe Ile Val
 195 200 205
 Asp Gly Arg Val Ser Leu Ser Val Ile Asp Gln Asp Gly Asp Val Ile
 210 215 220
 Pro Ala Arg Val Leu Glu Arg Gly Asp Phe Leu Gly Gln Thr Thr Leu
 225 230 235 240
 Thr Arg Glu Pro Val Leu Ala Thr Ala His Ala Leu Glu Glu Val Thr
 245 250 255
 Val Leu Glu Met Ala Arg Asp Glu Ile Glu Arg Leu Val His Arg Lys
 260 265 270
 Pro Ile Leu Leu His Val Ile Gly Ala Val
 275 280

<210> 135
 <211> 247
 <212> PRT
 <213> Mycobacterium vaccae

<400> 135
 Gly Tyr Gln Ser Gly Arg Ser Ser Leu Arg Ala Ser Val Phe Asp Arg
 1 5 10 15
 Leu Thr Asp Ile Arg Glu Ser Gln Ser Arg Gly Leu Glu Asn Gln Phe
 20 25 30
 Ala Asp Leu Lys Asn Ser Met Val Ile Tyr Ser Arg Gly Ser Thr Ala
 35 40 45
 Thr Glu Ala Ile Gly Ala Phe Ser Asp Gly Phe Arg Gln Leu Gly Asp
 50 55 60
 Ala Thr Ile Asn Thr Gly Gln Ala Ala Ser Leu Arg Arg Tyr Tyr Asp
 65 70 75 80
 Arg Thr Phe Ala Asn Thr Thr Leu Asp Asp Ser Gly Asn Arg Val Asp
 85 90 95
 Val Arg Ala Leu Ile Pro Lys Ser Asn Pro Gln Arg Tyr Leu Gln Ala
 100 105 110
 Leu Tyr Thr Pro Pro Phe Gln Asn Trp Glu Lys Ala Ile Ala Phe Asp
 115 120 125
 Asp Ala Arg Asp Gly Ser Ala Trp Ser Ala Ala Asn Ala Arg Phe Asn
 130 135 140
 Glu Phe Phe Arg Glu Ile Val His Arg Phe Asn Phe Glu Asp Leu Met
 145 150 155 160
 Leu Leu Asp Leu Glu Gly Asn Val Val Tyr Ser Ala Tyr Lys Gly Pro
 165 170 175
 Asp Leu Gly Thr Asn Ile Val Asn Gly Pro Tyr Arg Asn Arg Glu Leu
 180 185 190
 Ser Glu Ala Tyr Glu Lys Ala Val Ala Ser Asn Ser Ile Asp Tyr Val
 195 200 205
 Gly Val Thr Asp Phe Gly Trp Tyr Leu Pro Ala Glu Glu Pro Thr Ala
 210 215 220
 Trp Phe Leu Ser Pro Val Gly Leu Lys Asp Arg Val Asp Gly Val Met
 225 230 235 240
 Ala Val Gln Phe Pro Gly Ile
 245

<210> 136

<211> 45
 <212> DNA
 <213> Mycobacterium vaccae

<220>
 <221> unsure
 <222> (18) ... (18)

<400> 136
 atgagcgaaa tgcggcggncc ctggcggggtt ctggcatgtg gcatc

45

<210> 137
 <211> 340
 <212> DNA
 <213> Mycobacterium vaccae

<220>
 <221> unsure
 <222> (273) ... (273)

<221> unsure
 <222> (286) ... (286)

<400> 137
 gccaccggcg ggcgcgcgc ggtgcccgcc ggggtgagcg ccccgggcgt cgcgccggcc 60
 cccgcgatgc cgcggcgccc ggtgtccacg atcgcgccgg cgacctcggg caagctcagc 120
 gagtttttcg ccgccaaggg cgtcacgatg gagccgcagt ccagccgcga cttccgcgcc 180
 ctcaacatcg tgctgccgaa gccgcggggc tgggagcaca tcccggaacc gaacgtgccg 240
 gacgcgttcg cgggtgtggc cgaccgggtc agnggtaaag gtcagnagtc gacaaacgcc 300
 cacgtggttg tcgacaaaca cgtaggcgag ttcgacggca 340

<210> 138
 <211> 235
 <212> DNA
 <213> Mycobacterium vaccae

<220>
 <221> unsure
 <222> (16) ... (16)

<400> 138
 ggtgaccacc agcgtngaac aggtcgttgc cgaagccgcg gaggccaccg acgcgattgt 60
 caacggcttc aaggtcagcg ttccgggtcc ggggtccggc gcaccgccac ctgcaccggg 120
 tgccccgggt gtcccgcccg ccccgggcgc cccggcgctg ccgctggccg tcgcaccacc 180
 cccggctccc gctgttcccg ccgtggcgcc cgcgccacag ctgctgggac tgcag 235

<210> 139
 <211> 15
 <212> PRT
 <213> Mycobacterium vaccae

<400> 139
 Met Ser Glu Ile Ala Arg Pro Trp Arg Val Leu Ala Cys Gly Ile

1

5

10

15

<210> 140
 <211> 113
 <212> PRT
 <213> Mycobacterium vaccae

<220>
 <221> UNSURE
 <222> (96)...(96)

<400> 140
 Ala Thr Gly Gly Ala Ala Ala Val Pro Ala Gly Val Ser Ala Pro Ala
 1 5 10 15
 Val Ala Pro Ala Pro Ala Met Pro Ala Arg Pro Val Ser Thr Ile Ala
 20 25 30
 Pro Ala Thr Ser Gly Thr Leu Ser Glu Phe Phe Ala Ala Lys Gly Val
 35 40 45
 Thr Met Glu Pro Gln Ser Ser Arg Asp Phe Arg Ala Leu Asn Ile Val
 50 55 60
 Leu Pro Lys Pro Arg Gly Trp Glu His Ile Pro Asp Pro Asn Val Pro
 65 70 75 80
 Asp Ala Phe Ala Val Leu Ala Asp Arg Val Gly Gly Lys Gly Gln Xaa
 85 90 95
 Ser Thr Asn Ala His Val Val Val Asp Lys His Val Gly Glu Phe Asp
 100 105 110
 Gly

<210> 141
 <211> 73
 <212> PRT
 <213> Mycobacterium vaccae

<400> 141
 Val Thr Thr Ser Val Glu Gln Val Val Ala Ala Ala Asp Ala Thr Glu
 1 5 10 15
 Ala Ile Val Asn Gly Phe Lys Val Ser Val Pro Gly Pro Gly Pro Ala
 20 25 30
 Ala Pro Pro Pro Ala Pro Gly Ala Pro Gly Val Pro Pro Ala Pro Gly
 35 40 45
 Ala Pro Ala Leu Pro Leu Ala Val Ala Pro Pro Pro Ala Pro Ala Val
 50 55 60
 Pro Ala Val Ala Pro Ala Pro Gln Leu
 65 70

<210> 142
 <211> 273
 <212> DNA
 <213> Mycobacterium vaccae

<400> 142
 ggcacctacg tgcagggggg tctcggccgc atcgaggccc ggggtggccga cagcggatac

60

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agcaacgccg cggccaaggg ctacttcccc ctgagcttca ccgtcgccgg catcgaccag 120
aacgggtccga tcgtgaccgc caacgtcacc gcggcgcccc cgacggggcg cgtggccacc 180
cagccgctga cgttcacgcg cgggcccagc ccgaccggat ggcagctgtc caagcagtc 240
gcactggccc tgatgtccgc ggtcatcgcc gca 273

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<210> 143
<211> 91
<212> PRT
<213> Mycobacterium vaccae

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<400> 143
Ala Thr Tyr Val Gln Gly Gly Leu Gly Arg Ile Glu Ala Arg Val Ala
 1          5          10          15
Asp Ser Gly Tyr Ser Asn Ala Ala Lys Gly Tyr Phe Pro Leu Ser
 20          25          30
Phe Thr Val Ala Gly Ile Asp Gln Asn Gly Pro Ile Val Thr Ala Asn
 35          40          45
Val Thr Ala Ala Ala Pro Thr Gly Ala Val Ala Thr Gln Pro Leu Thr
 50          55          60
Phe Ile Ala Gly Pro Ser Pro Thr Gly Trp Gln Leu Ser Lys Gln Ser
 65          70          75          80
Ala Leu Ala Leu Met Ser Ala Val Ile Ala Ala
          85          90

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<210> 144
<211> 554
<212> DNA
<213> Mycobacterium vaccae

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<400> 144
gatgtcacgc ccggagaatg taacgttcga ccggagaacg ccgtcggcac aacgagttac 60
gtttgagcac ttcagatctc gggtaccttg gatttcaggc gggggaagca gtaaccgatc 120
caagattcga aggacccaaa caacatgaaa ttacttgaa tgaccgtgcg cgcaagcccg 180
gcgccctggc cggcgctcggg gcggcatgtc tgttcggcgg cgtggcccg gcaaccgtgg 240
cggcacagat ggccggcgcc cagccggccg agtgcaacgc cagctcactc accggcaccg 300
tcagctcggg gaccgggtcag gcgcgtcagt acctagacac ccaccggggc gccaaaccagg 360
ccgtcaccgc ggcgatgaac cagccgcggc ccgaggccga ggcgaacctg cggggctact 420
tcaccgcaa cccggcgagg tactacgacc tgccgggcat cctcgccccg atcggtgacg 480
cgcagcgcaa ctgcaacatc accgtgctgc cggtagagct gcagacggcc tacgacacgt 540
tcatggccgg ctga 554

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<210> 145
<211> 136
<212> PRT
<213> Mycobacterium vaccae

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```

<400> 145
Met Lys Phe Thr Gly Met Thr Val Arg Ala Ser Arg Arg Ala Leu Ala
 1          5          10          15
Gly Val Gly Ala Ala Cys Leu Phe Gly Gly Val Ala Ala Ala Thr Val
 20          25          30
Ala Ala Gln Met Ala Gly Ala Gln Pro Ala Glu Cys Asn Ala Ser Ser
 35          40          45

```


Leu Thr Gly Thr Val Ser Ser Val Thr Gly Gln Ala Arg Gln Tyr Leu
 50 55 60
 Asp Thr His Pro Gly Ala Asn Gln Ala Val Thr Ala Ala Met Asn Gln
 65 70 75 80
 Pro Arg Pro Glu Ala Ala Asn Leu Arg Gly Tyr Phe Thr Ala Asn
 85 90 95
 Pro Ala Glu Tyr Tyr Asp Leu Arg Gly Ile Leu Ala Pro Ile Gly Asp
 100 105 110
 Ala Gln Arg Asn Cys Asn Ile Thr Val Leu Pro Val Glu Leu Gln Thr
 115 120 125
 Ala Tyr Asp Thr Phe Met Ala Gly
 130 135

<210> 146
 <211> 808
 <212> DNA
 <213> Mycobacterium vaccae

<220>
 <221> unsure
 <222> (15) ... (15)

<400> 146
 ccaagtgtga cgcgngtgtg acggtagacg ttccgaccaa tccaacgacg ccgcagctgg 60
 gaatcacccg tgtgcccaatt cagtgcgggc aacgggtgtcc gtccacgaag ggattcagga 120
 aatgatgaca actcgccgga agtcagccgc agtggcgagg atcgctgcgg tggccatcct 180
 cgggtgcggcc gcatgttcga gtgaggacgg tgggagcacg gcctcgtcgg ccagcagcac 240
 ggctcctcc gcgatggagt ccgcgaccga cgagatgacc acgtcgtcgg cggccccctc 300
 ggccgaccct gcggccaacc tgatcggtc cggtgcgcg gcctacgccg agcaggtccc 360
 cgaaggcccc gggtcggtgg ccgggatggc agccgatccg gtgacggtgg cggcgtcgaa 420
 caaccgatg ctgcagacgc tgtcccaggc gctgtccggc cagctcaatc cgcaggtcaa 480
 tctcgtcgac accctcgacg gcggtgagtt caccgtgttc gcgcgaccg acgacgcgtt 540
 cgccaagatc gatccggcca cgctggagac cctcaagacg gactccgaca tgctgaccaa 600
 catcctgacc taccacgtcg tgcccggcca ggccgcgccc gatcaggtgg tcggcgagca 660
 tgtgacggtg gagggggcgc cggtcacggt gtccgggatg gccgaccagc tcaaggtcaa 720
 cgacgcgtcg gtgggtgtgcg gtgggggtgca gaccgccaac gcgacggtgt atctgatcga 780
 caccgtgctg atgcgcgccg cagcgtag 808

<210> 147
 <211> 228
 <212> PRT
 <213> Mycobacterium vaccae

<400> 147
 Met Met Thr Thr Arg Arg Lys Ser Ala Ala Val Ala Gly Ile Ala Ala
 1 5 10 15
 Val Ala Ile Leu Gly Ala Ala Ala Cys Ser Ser Glu Asp Gly Gly Ser
 20 25 30
 Thr Ala Ser Ser Ala Ser Ser Thr Ala Ser Ser Ala Met Glu Ser Ala
 35 40 45
 Thr Asp Glu Met Thr Thr Ser Ser Ala Ala Pro Ser Ala Asp Pro Ala
 50 55 60
 Ala Asn Leu Ile Gly Ser Gly Cys Ala Ala Tyr Ala Glu Gln Val Pro

65		70		75		80
Glu Gly Pro Gly Ser Val Ala Gly Met Ala Ala Asp Pro Val Thr Val						
	85		90		95	
Ala Ala Ser Asn Asn Pro Met Leu Gln Thr Leu Ser Gln Ala Leu Ser						
	100		105		110	
Gly Gln Leu Asn Pro Gln Val Asn Leu Val Asp Thr Leu Asp Gly Gly						
	115		120		125	
Glu Phe Thr Val Phe Ala Pro Thr Asp Asp Ala Phe Ala Lys Ile Asp						
	130		135		140	
Pro Ala Thr Leu Glu Thr Leu Lys Thr Asp Ser Asp Met Leu Thr Asn						
145		150		155		160
Ile Leu Thr Tyr His Val Val Pro Gly Gln Ala Ala Pro Asp Gln Val						
	165		170		175	
Val Gly Glu His Val Thr Val Glu Gly Ala Pro Val Thr Val Ser Gly						
	180		185		190	
Met Ala Asp Gln Leu Lys Val Asn Asp Ala Ser Val Val Cys Gly Gly						
	195		200		205	
Val Gln Thr Ala Asn Ala Thr Val Tyr Leu Ile Asp Thr Val Leu Met						
	210		215		220	
Pro Pro Ala Ala						
225						

<210> 148
 <211> 22
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Made in a lab

<221> unsure
 <222> (12)...(12)

<221> unsure
 <222> (17)...(17)

<400> 148
 gcscscgtsg gnccggntgy gc

22

<210> 149
 <211> 21
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Made in a lab

<221> unsure
 <222> (10)...(10)

<221> unsure
 <222> (13)...(13)

<221> unsure
<222> (16) ... (16)

<221> unsure
<222> (20) ... (20)

<400> 149
rtasgcsgcn gtngcnacng g

21

<210> 150
<211> 102
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 150
gcccccgctcg gccccggctg tgcggcctac gtgcaacagg tgccggacgg gccgggatcg 60
gtgcaggggca tggcgagctc gcccgtagcg accgccgcgt at 102

<210> 151
<211> 683
<212> DNA
<213> Mycobacterium vaccae

<400> 151
gccccccaac taaaaccgcc gatcatccac tgcaggaagg aatctcacga tcatgaacat 60
cagcatgaaa actcttgccg gagggggttt cgcgatgacc gccgccgtcg gtctgtcgct 120
gggtaccgca ggcagcgccg cagccgcgcc ggctcgaccg ggggtgtgcgg cctacgtgca 180
acaggtgccg gacggggccgg gatcggtgca gggcatggcg agctcgccgg tggccaccgc 240
ggcggccgac aaccgcgtgc tcaccacgct ctgcgaggcg atctcgggtc agctcaaccc 300
gaacgtcaat ctctcgacac cgttcaacgg cggccagttc accgtgttcg cgccgaccaa 360
tgacgccttc gccaaagatcg atccggccac gctggagacc ctcaagaccg attccgacct 420
gctgaccaag atcctcacct accacgtcgt gcccggccag gccgcgcccg atcaggtggt 480
cggcgagcat gtgacgggtg agggggcgcc ggtcacgggtg tccgggatgg ccgaccagct 540
caaggtcaac gacgcgtcgg tgggtgtcgg tgggggtcag accgccaacg cgacgggtgta 600
tctgatcgac accgtgctga tgccgccggc agcgtagccg ggcggcacca cagaagaggg 660
tccccgcac ccggcctccc ccg 683

<210> 152
<211> 231
<212> PRT
<213> Mycobacterium vaccae

<400> 152
Asp Thr Val Leu Met Pro Pro Ala Asn Asn Arg Arg Ser Ser Thr Ala
1 5 10 15
Gly Arg Asn Leu Thr Ile Met Asn Ile Ser Met Lys Thr Leu Ala Gly
20 25 30
Ala Gly Phe Ala Met Thr Ala Ala Val Gly Leu Ser Leu Gly Thr Ala
35 40 45
Gly Ser Ala Ala Ala Pro Val Gly Pro Gly Cys Ala Ala Tyr Val

50	55	60
Gln Gln Val Pro Asp Gly Pro Gly Ser Val Gln Gly Met Ala Ser Ser		
65	70	75
Pro Val Ala Thr Ala Ala Asp Asn Pro Leu Leu Thr Thr Leu Ser		80
	85	90
Gln Ala Ile Ser Gly Gln Leu Asn Pro Asn Val Asn Leu Val Asp Thr		95
	100	105
Phe Asn Gly Gly Gln Phe Thr Val Phe Ala Pro Thr Asn Asp Ala Phe		110
	115	120
Ala Lys Ile Asp Pro Ala Thr Leu Glu Thr Leu Lys Thr Asp Ser Asp		125
	130	135
Leu Leu Thr Lys Ile Leu Thr Tyr His Val Val Pro Gly Gln Ala Ala		140
145	150	155
Pro Asp Gln Val Val Gly Glu His Val Thr Val Glu Gly Ala Pro Val		160
	165	170
Thr Val Ser Gly Met Ala Asp Gln Leu Lys Val Asn Asp Ala Ser Val		175
	180	185
Val Cys Gly Gly Val Gln Thr Ala Asn Ala Thr Val Tyr Leu Ile Asp		190
	195	200
Thr Val Leu Met Pro Pro Ala Ala Pro Gly Gly Thr Thr Glu Glu Gly		205
	210	215
Pro Pro His Pro Ala Ser Pro		220
225	230	

<210> 153

<211> 1125

<212> DNA

<213> Mycobacterium vaccae

<220>

<221> unsure

<222> (358)...(358)

<400> 153

atgcaggtgc	ggcgtgttct	gggcagtgtc	ggtgcagcag	tcgcggtttc	ggccgcgtta	60
tggcagacgg	gggtttcgat	accgaccgcc	tcagcggatc	cgtgtccgga	catcgaggtg	120
atcttcgcgc	gcgggaccgg	tgcggaaccc	ggcctcgggt	gggtcgggtga	tgcgttcgtc	180
aacgcgctgc	ggcccaaggt	cggtgagcag	tcggtgggca	cctacgcggt	gaactaccgc	240
gcaggattcg	gacttcgaca	aatcggcgcc	catgggcgcg	gccgacgcat	cggggcgggt	300
gcagtggatg	gccgacaact	gcccggacac	caagcttgtc	ctgggcggca	tgtcgcangg	360
cgccggcgtc	atcgacctga	tcaccgtcga	tccgcgaccg	ctgggcgggt	tcacccccac	420
cccgatgccg	ccccgcgtcg	ccgaccacgt	ggccgcgggt	gtggtcttcg	gaaatccggt	480
gcgcgacatc	cgtggtggcg	gtccgctgcc	gcagatgagc	ggcacctacg	ggccgaagtc	540
gatcgatctg	tgtgcgctcg	acgatccggt	ctgctcgccc	ggcttcaacc	tgccggccca	600
cttcgcctac	gccgacaacg	gcatggtgga	ggaagccgcg	aacttcgccc	gcctggaacc	660
gggcccagagc	gtcgagctgc	ccgaggcgcc	ctacctgcac	ctgttcgtcc	cgcggggcga	720
ggtaacgctg	gaggacgcgc	gaccgctgcg	cgaaggcgac	gcagtgcgtt	tcaccgcatac	780
gggcggccag	cgggtgaccg	ccaccgcgcc	cgcggagatc	ctcgtctggg	agatgcatgc	840
gggactcggt	gcggcataag	cgaataggag	tcctgctggc	cggcgcagca	ctgctcgccg	900
gatgcacatc	cgaacctgga	cccggggccg	cggcggcacc	ggccccgacg	agcacaaccg	960
agagcgcacc	cgggtcccga	ctcggtcccg	tgaccgtcgc	ggtcgacgaa	cctctggccg	1020
acgcgccggt	cgaccagccc	cgggaggccc	tggtgcccga	gggttgacg	ctgtcggtgt	1080
gggcgcggac	cgcccgccg	cggctggccg	cgtgggcccc	ggacg		1125

<210> 154
 <211> 748
 <212> PRT
 <213> Mycobacterium vaccae

<220>
 <221> UNSURE
 <222> (119)... (119)

<400> 154
 Met Gln Val Arg Arg Val Leu Gly Ser Val Gly Ala Ala Val Ala Val
 1 5 10 15
 Ser Ala Ala Leu Trp Gln Thr Gly Val Ser Ile Pro Thr Ala Ser Ala
 20 25 30
 Asp Pro Cys Pro Asp Ile Glu Val Ile Phe Ala Arg Gly Thr Gly Ala
 35 40 45
 Glu Pro Gly Leu Gly Trp Val Gly Asp Ala Phe Val Asn Ala Leu Arg
 50 55 60
 Pro Lys Val Gly Glu Gln Ser Val Gly Thr Tyr Ala Val Asn Tyr Pro
 65 70 75 80
 Ala Gly Phe Asp Phe Asp Lys Ser Ala Pro Met Gly Ala Ala Asp Ala
 85 90 95
 Ser Gly Arg Val Gln Trp Met Ala Asp Asn Cys Pro Asp Thr Lys Leu
 100 105 110
 Val Leu Gly Gly Met Ser Xaa Gly Ala Gly Val Ile Asp Leu Ile Thr
 115 120 125
 Val Asp Pro Arg Pro Leu Gly Arg Phe Thr Pro Thr Pro Met Pro Pro
 130 135 140
 Arg Val Ala Asp His Val Ala Ala Val Val Val Phe Gly Asn Pro Leu
 145 150 155 160
 Arg Asp Ile Arg Gly Gly Gly Pro Arg Leu Glu Pro Arg Gly Leu Asn
 165 170 175
 Met Glu Thr Ser Glu Arg Gly Leu Tyr Thr His Arg Thr Tyr Arg Gly
 180 185 190
 Leu Tyr Pro Arg Leu Tyr Ser Ser Glu Arg Ile Leu Glu Ala Ser Pro
 195 200 205
 Leu Glu Cys Tyr Ser Ala Leu Ala Leu Glu Ala Ser Pro Ala Ser Pro
 210 215 220
 Pro Arg Pro His Glu Cys Tyr Ser Ser Glu Arg Pro Arg Gly Leu Tyr
 225 230 235 240
 Pro His Glu Ala Ser Asn Leu Glu Pro Arg Ala Leu Ala His Ile Ser
 245 250 255
 Pro His Glu Ala Leu Ala Thr Tyr Arg Ala Leu Ala Ala Ser Pro Ala
 260 265 270
 Ser Asn Gly Leu Tyr Met Glu Thr Val Ala Leu Gly Leu Gly Leu Ala
 275 280 285
 Leu Ala Ala Leu Ala Ala Ser Asn Pro His Glu Ala Leu Ala Ala Arg
 290 295 300
 Gly Leu Glu Gly Leu Pro Arg Gly Leu Tyr Gly Leu Asn Ser Glu Arg
 305 310 315 320
 Val Ala Leu Gly Leu Leu Glu Pro Arg Gly Leu Ala Leu Ala Pro Arg
 325 330 335

Thr Tyr Arg Leu Glu His Ile Ser Leu Glu Pro His Glu Val Ala Leu
 340 345 350
 Pro Arg Ala Arg Gly Gly Leu Tyr Gly Leu Val Ala Leu Thr His Arg
 355 360 365
 Leu Glu Gly Leu Ala Ser Pro Ala Leu Ala Gly Leu Tyr Pro Arg Leu
 370 375 380
 Glu Ala Arg Gly Gly Leu Gly Leu Tyr Ala Ser Pro Ala Leu Ala Val
 385 390 395 400
 Ala Leu Ala Arg Gly Pro His Glu Thr His Arg Ala Leu Ala Ser Glu
 405 410 415
 Arg Gly Leu Tyr Gly Leu Tyr Gly Leu Asn Ala Arg Gly Val Ala Leu
 420 425 430
 Thr His Arg Ala Leu Ala Thr His Arg Ala Leu Ala Pro Arg Ala Leu
 435 440 445
 Ala Gly Leu Ile Leu Glu Leu Glu Val Ala Leu Thr Arg Pro Gly Leu
 450 455 460
 Met Glu Thr His Ile Ser Ala Leu Ala Gly Leu Tyr Leu Glu Gly Leu
 465 470 475 480
 Tyr Ala Leu Ala Ala Leu Ala Ala Leu Ala Ala Ser Asn Ala Arg Gly
 485 490 495
 Ser Glu Arg Pro Arg Ala Leu Ala Gly Leu Tyr Ala Arg Gly Ala Arg
 500 505 510
 Gly Ser Glu Arg Thr His Arg Ala Leu Ala Ala Arg Gly Ala Arg Gly
 515 520 525
 Met Glu Thr His Ile Ser Ile Leu Glu Ala Arg Gly Thr His Arg Thr
 530 535 540
 Arg Pro Thr His Arg Ala Arg Gly Ala Leu Ala Val Ala Leu Gly Leu
 545 550 555 560
 Tyr Gly Leu Tyr Thr His Arg Gly Leu Tyr Pro Arg Ala Ser Pro Gly
 565 570 575
 Leu His Ile Ser Ala Ser Asn Ala Arg Gly Gly Leu Ala Arg Gly Thr
 580 585 590
 His Arg Ala Arg Gly Ser Glu Arg Ala Arg Gly Thr His Arg Ala Arg
 595 600 605
 Gly Pro Arg Gly Leu Tyr Ala Ser Pro Ala Arg Gly Ala Arg Gly Gly
 610 615 620
 Leu Tyr Ala Arg Gly Ala Arg Gly Thr His Arg Ser Glu Arg Gly Leu
 625 630 635 640
 Tyr Ala Arg Gly Ala Arg Gly Ala Leu Ala Val Ala Leu Ala Arg Gly
 645 650 655
 Pro Arg Ala Leu Ala Pro Arg Gly Leu Tyr Gly Leu Tyr Pro Arg Gly
 660 665 670
 Leu Tyr Ala Leu Ala Ala Leu Ala Gly Leu Tyr Leu Glu Ala Ser Pro
 675 680 685
 Ala Leu Ala Val Ala Leu Gly Leu Tyr Val Ala Leu Gly Leu Tyr Ala
 690 695 700
 Leu Ala Ala Ser Pro Ala Arg Gly Pro Arg Ala Leu Ala Ala Leu Ala
 705 710 715 720
 Ala Leu Ala Gly Leu Tyr Ala Arg Gly Val Ala Leu Gly Leu Tyr Pro
 725 730 735
 Arg Gly Leu Tyr Ala Arg Gly Pro Arg Gly Leu Tyr
 740 745

<210> 155
 <211> 666
 <212> DNA
 <213> Mycobacterium vaccae

<400> 155
 atgaaggcaa atcattcggg atgctacaaa tccgccggcc cgatatggtc gcatccatcg 60
 ccgctttgtt cgcccgcact ggcaccatct catgcaggtc tggacaatga gctgagcctg 120
 ggcattccacg gccagggccc ggaacgactg accattcagc agtgggacac cttcctcaac 180
 ggcgctcttc cgttggaccg caaccggttg acccgggagt ggttccactc gggcaaggcg 240
 acctacgtcg tggccgggtga aggtgccgac gagttcgagg gcacgctgga gctggggtac 300
 caggtgggct ttccgtggtc gctgggcgtg ggcattcaact tcagctacac caccgccaac 360
 atcacgtacg acggttacgg cctcaacttc gccgaccgc tgetgggctt cgggtgattcc 420
 atcgtgacct cgccgctgtt cccgggtgtc tcatcacgg cggacctggg caacggcccc 480
 ggcatccagg aggtcgcgac cttctccgtg gacgtggccg gcccgggtgg ttccgtgggtg 540
 gtgtccaacg cgcacggcac ggtcaccggt gctgccggtg gtgtgctgct gcgtccgttc 600
 gccgcctga tctcgtcgac cggcgacagc gtcaccacct acggcgcacc ctggaacatg 660
 aactga 666

<210> 156
 <211> 221
 <212> PRT
 <213> Mycobacterium vaccae

<400> 156
 Met Lys Ala Asn His Ser Gly Cys Tyr Lys Ser Ala Gly Pro Ile Trp
 1 5 10 15
 Ser His Pro Ser Pro Leu Cys Ser Pro Ala Leu Ala Pro Ser His Ala
 20 25 30
 Gly Leu Asp Asn Glu Leu Ser Leu Gly Val His Gly Gln Gly Pro Glu
 35 40 45
 His Leu Thr Ile Gln Gln Trp Asp Thr Phe Leu Asn Gly Val Phe Pro
 50 55 60
 Leu Asp Arg Asn Arg Leu Thr Arg Glu Trp Phe His Ser Gly Lys Ala
 65 70 75 80
 Thr Tyr Val Val Ala Gly Glu Gly Ala Asp Glu Phe Glu Gly Thr Leu
 85 90 95
 Glu Leu Gly Tyr His Val Gly Phe Pro Trp Ser Leu Gly Val Gly Ile
 100 105 110
 Asn Phe Ser Tyr Thr Thr Pro Asn Ile Thr Tyr Asp Gly Tyr Gly Leu
 115 120 125
 Asn Phe Ala Asp Pro Leu Leu Gly Phe Gly Asp Ser Ile Val Thr Pro
 130 135 140
 Pro Leu Phe Pro Gly Val Ser Ile Thr Ala Asp Leu Gly Asn Gly Pro
 145 150 155 160
 Gly Ile Gln Glu Val Ala Thr Phe Ser Val Asp Val Ala Gly Pro Gly
 165 170 175
 Gly Ser Val Val Val Ser Asn Ala His Gly Thr Val Thr Gly Ala Ala
 180 185 190
 Gly Gly Val Leu Leu Arg Pro Phe Ala Arg Leu Ile Ser Ser Thr Gly
 195 200 205
 Asp Ser Val Thr Thr Tyr Gly Ala Pro Trp Asn Met Asn
 210 215 220

<210> 157
 <211> 480
 <212> DNA
 <213> Mycobacterium vaccae

<400> 157
 aacggctggg acatcaacac ccctgcgttc gagtggttct acgagtcagg cttgtcgacg 60
 atcatgccgg tcggcggaaca gtccagcttc tacagcgact ggtaccagcc gtctcggggc 120
 aacgggcaga actacaccta caagtgggag acgttcctga cccaggagct gccgacgtgg 180
 ctggaggcca accgcggagt gtcgcgcacc ggcaacgcgt tcgtcggcct gtcgatggcg 240
 ggcagcgagg cgctgacctt cgcgatccat caccgcgagc agttcatcta cgcctcgtcg 300
 ctgtcaggct tcctgaaccc gtccgagggc tgggtggcga tgctgatcgg gctggcgatg 360
 aacgacgcag gcgggttcaa cgccgagagc atgtggggcc cgtcctcgga cccggcgatg 420
 aagcgcaacg acccgatggt caacatcaac cagctgggtg ccaacaacac ccggatctgg 480

<210> 158
 <211> 161
 <212> PRT
 <213> Mycobacterium vaccae

<400> 158
 Asn Gly Trp Asp Ile Asn Thr Pro Ala Phe Glu Trp Phe Tyr Glu Ser
 1 5 10 15
 Gly Leu Ser Thr Ile Met Pro Val Gly Gly Gln Ser Ser Phe Tyr Ser
 20 25 30
 Asp Trp Tyr Gln Pro Ser Arg Gly Asn Gly Gln Asn Tyr Thr Tyr Lys
 35 40 45
 Trp Glu Thr Phe Leu Thr Gln Glu Leu Pro Thr Trp Leu Glu Ala Asn
 50 55 60
 Arg Gly Val Ser Arg Thr Gly Asn Ala Phe Val Gly Leu Ser Met Ala
 65 70 75 80
 Gly Ser Ala Ala Leu Thr Tyr Ala Ile His His Pro Gln Gln Phe Ile
 85 90 95
 Tyr Ala Ser Ser Leu Ser Gly Phe Leu Asn Pro Ser Glu Gly Trp Trp
 100 105 110
 Pro Met Leu Ile Gly Leu Ala Met Asn Asp Ala Gly Gly Phe Asn Ala
 115 120 125
 Glu Ser Met Trp Gly Pro Ser Ser Asp Pro Ala Trp Lys Arg Asn Asp
 130 135 140
 Pro Met Val Asn Ile Asn Gln Leu Val Ala Asn Asn Thr Arg Ile Trp
 145 150 155 160
 Ile

<210> 159
 <211> 1626
 <212> DNA
 <213> Mycobacterium vaccae

<400> 159
 atggccaaga caattgcgta tgacgaagag gcccgcggtg gctcgcgagc gggcctcaac 60
 gccctcgcag acgccgtaaa ggtgacgttg ggcccgaagg gtcgcaacgt cgtgctggag 120


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aagaagtggg ggcggccac gatcaccaac gatggtgtgt ccatcgccaa ggagatcgag 180
ctggaggacc cgtacgagaa gatcggcgct gagctggtca aagaggtcgc caagaagacc 240
gacgacgtcg cgggcgacgg caccaccacc gccaccgtgc tcgctcaggc tctggttcgc 300
gaaggcctgc gcaacgtcgc agccggcgcc aaccgcgtcg gcctcaagcg tggcatcgag 360
aaggctgtcg aggtgtgcac ccagtcgctg ctgaagtcgg ccaaggaggt cgagaccaag 420
gagcagattt ctgccaccgc ggcgatttcc gccggcgaca cccagatcgg cgagctcatc 480
gccgaggcca tggacaaggt cggcaacgag ggtgtcatca ccgtcgagga gtcgaacacc 540
ttcggcctgc agctcgagct caccgagggt atgcgcttcg acaagggcta catctcgggt 600
tacttcgtga ccgacgccga gcgccaggaa gccgtcctgg aggatcccta catcctgctg 660
gtcagctcca aggtgtcgac cgtcaaggat ctgctcccgc tgctggagaa ggtcatccag 720
gccggcaagc cgctgtgat catcgccgag gacgtcgagg gcgaggccct gtccacgctg 780
gtggtcaaca agatccgcgg caccttcaag tccgtcgccg tcaaggctcc gggcttcgggt 840
gaccgccgca aggcgatgct gcaggacatg gccatcctca ccggtggtca ggtcgtcagc 900
gaaagagtcg ggctgtccct ggagaccgc gacgtctcgc tgctgggcca ggcccgcaag 960
gtcgtcgtca ccaaggacga gaccaccatc gtcgagggct cgggcgattc cgatgccatc 1020
gccggccggg tggctcagat ccgcgccgag atcgagaaca gcgactccga ctacgaccgc 1080
gagaagctgc aggagcgctt ggccaagctg gccggcggtg ttgcggtgat caaggccgga 1140
gctgccaccg aggtggagct caaggagcgc aagcaccgca tcgaggacgc cgtccgcaac 1200
gcgaaggctg ccgtcgaaga gggcatcgtc gccggtggcg gcgtggctct gctgcagtcg 1260
gtcctcgccg tggacgacct cggcctgacg ggcgacgagg ccaccggtgc caacatcgtc 1320
cgcggtggcg tgctcggtcc gctcaagcag atcgcttca acggcgccct ggagcccggc 1380
gtcgttgccg agaaggtgtc caacctgccc gcgggtcacg gcctcaacgc cgcgaccggt 1440
gagtacgagg acctgtcaa ggcggcgctc gccgaccggt tgaaggtcac ccgctcggcg 1500
ctgcagaacg cggcgctccat cgcggctctg ttcctacca ccgaggccgt cgtcgccgac 1560
aagccggaga aggcgtccgc acccgcgggc gacccgaccg gtggcatggg cggtatggac 1620
ttctaa 1626

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<210> 160

<211> 541

<212> PRT

<213> *Mycobacterium vaccae*

<400> 160

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Met Ala Lys Thr Ile Ala Tyr Asp Glu Glu Ala Arg Arg Gly Leu Glu
 1           5           10           15
Arg Gly Leu Asn Ala Leu Ala Asp Ala Val Lys Val Thr Leu Gly Pro
          20           25           30
Lys Gly Arg Asn Val Val Leu Glu Lys Lys Trp Gly Ala Pro Thr Ile
          35           40           45
Thr Asn Asp Gly Val Ser Ile Ala Lys Glu Ile Glu Leu Glu Asp Pro
          50           55           60
Tyr Glu Lys Ile Gly Ala Glu Leu Val Lys Glu Val Ala Lys Lys Thr
        65           70           75           80
Asp Asp Val Ala Gly Asp Gly Thr Thr Thr Ala Thr Val Leu Ala Gln
          85           90           95
Ala Leu Val Arg Glu Gly Leu Arg Asn Val Ala Ala Gly Ala Asn Pro
          100          105          110
Leu Gly Leu Lys Arg Gly Ile Glu Lys Ala Val Glu Ala Val Thr Gln
          115          120          125
Ser Leu Leu Lys Ser Ala Lys Glu Val Glu Thr Lys Glu Gln Ile Ser
          130          135          140
Ala Thr Ala Ala Ile Ser Ala Gly Asp Thr Gln Ile Gly Glu Leu Ile
          145          150          155          160

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Ala Glu Ala Met Asp Lys Val Gly Asn Glu Gly Val Ile Thr Val Glu
 165 170 175
 Glu Ser Asn Thr Phe Gly Leu Gln Leu Glu Leu Thr Glu Gly Met Arg
 180 185 190
 Phe Asp Lys Gly Tyr Ile Ser Gly Tyr Phe Val Thr Asp Ala Glu Arg
 195 200 205
 Gln Glu Ala Val Leu Glu Asp Pro Tyr Ile Leu Leu Val Ser Ser Lys
 210 215 220
 Val Ser Thr Val Lys Asp Leu Leu Pro Leu Leu Glu Lys Val Ile Gln
 225 230 235 240
 Ala Gly Lys Pro Leu Leu Ile Ile Ala Glu Asp Val Glu Gly Glu Ala
 245 250 255
 Leu Ser Thr Leu Val Val Asn Lys Ile Arg Gly Thr Phe Lys Ser Val
 260 265 270
 Ala Val Lys Ala Pro Gly Phe Gly Asp Arg Arg Lys Ala Met Leu Gln
 275 280 285
 Asp Met Ala Ile Leu Thr Gly Gly Gln Val Val Ser Glu Arg Val Gly
 290 295 300
 Leu Ser Leu Glu Thr Ala Asp Val Ser Leu Leu Gly Gln Ala Arg Lys
 305 310 315 320
 Val Val Val Thr Lys Asp Glu Thr Thr Ile Val Glu Gly Ser Gly Asp
 325 330 335
 Ser Asp Ala Ile Ala Gly Arg Val Ala Gln Ile Arg Ala Glu Ile Glu
 340 345 350
 Asn Ser Asp Ser Asp Tyr Asp Arg Glu Lys Leu Gln Glu Arg Leu Ala
 355 360 365
 Lys Leu Ala Gly Gly Val Ala Val Ile Lys Ala Gly Ala Ala Thr Glu
 370 375 380
 Val Glu Leu Lys Glu Arg Lys His Arg Ile Glu Asp Ala Val Arg Asn
 385 390 395 400
 Ala Lys Ala Ala Val Glu Glu Gly Ile Val Ala Gly Gly Gly Val Ala
 405 410 415
 Leu Leu Gln Ser Ala Pro Ala Leu Asp Asp Leu Gly Leu Thr Gly Asp
 420 425 430
 Glu Ala Thr Gly Ala Asn Ile Val Arg Val Ala Leu Ser Ala Pro Leu
 435 440 445
 Lys Gln Ile Ala Phe Asn Gly Gly Leu Glu Pro Gly Val Val Ala Glu
 450 455 460
 Lys Val Ser Asn Leu Pro Ala Gly His Gly Leu Asn Ala Ala Thr Gly
 465 470 475 480
 Glu Tyr Glu Asp Leu Leu Lys Ala Gly Val Ala Asp Pro Val Lys Val
 485 490 495
 Thr Arg Ser Ala Leu Gln Asn Ala Ala Ser Ile Ala Ala Leu Phe Leu
 500 505 510
 Thr Thr Glu Ala Val Val Ala Asp Lys Pro Glu Lys Ala Ser Ala Pro
 515 520 525
 Ala Gly Asp Pro Thr Gly Gly Met Gly Gly Met Asp Phe
 530 535 540

<210> 161

<211> 985

<212> DNA

<213> Mycobacterium vaccae

<400> 161

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ggatccctac atcctgctgg tcagctccaa ggtgtcgacc gtcaaggatc tgctcccgt      60
gctggagaag gtcattcagg ccggcaagcc gctgctgac atcgccgagg acgtcgaggg      120
cgaggccctg tccacgctgg tggtaacaa gatccgcggc accttcaagt ccgtcgccgt      180
caaggctccg ggcttcggtg accgccgcaa ggcgatgctg caggacatgg ccatcctcac      240
cgggtggtcag gtcgtcagcg aaagagtcgg gctgtccctg gagaccgccg acgtctcgct      300
gctggggccag gcccgcaagg tcgtcgtcac caaggacgag accaccatcg tcgagggctc      360
ggggcattcc gatgccatcg ccggccgggt ggtcagatc cgcgccgaga tcgagaacag      420
cgactccgac tacgaccgcg agaagctgca ggagcgctg gccaaagtgg ccggcgggtg      480
tgcggtgatc aaggccggag ctgccaccga ggtggagctc aaggagcgca agcaccgcat      540
cgaggacgcc gtccgcaacg cgaaggctgc cgtcgaagag ggcacgtcg ccggtggcgg      600
cgtggctctg ctgcagtcgg ctctgctgct ggacgacctg ggcctgacgg gcgacgaggg      660
caccggtgccc aacatcgctc gcgtggcgct gtcggctccg ctcaagcaga tcgccttcaa      720
cggcgccctg gagcccgcg tcgttgccga gaagggtgtc aacctgccg ccggttcagg      780
cctcaacgcc gcgaccggtg agtacgagga cctgctcaag gccggcgctc ccgaccgggt      840
gaaggtcacc cgctcggcgc tgcagaacgc ggcgtccatc gcggctctgt tctcaccac      900
cgaggccgct gtcgccgaca agccggagaa ggcgtccgca cccgcggggc acccgaccgg      960
tggcatgggc ggtatggact tctaa                                     985

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<210> 162

<211> 327

<212> PRT

<213> Mycobacterium vaccae

<400> 162

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Asp Pro Tyr Ile Leu Leu Val Ser Ser Lys Val Ser Thr Val Lys Asp
1      5      10      15
Leu Leu Pro Leu Leu Glu Lys Val Ile Gln Ala Gly Lys Pro Leu Leu
20     25     30
Ile Ile Ala Glu Asp Val Glu Gly Glu Ala Leu Ser Thr Leu Val Val
35     40     45
Asn Lys Ile Arg Gly Thr Phe Lys Ser Val Ala Val Lys Ala Pro Gly
50     55     60
Phe Gly Asp Arg Arg Lys Ala Met Leu Gln Asp Met Ala Ile Leu Thr
65     70     75     80
Gly Gly Gln Val Val Ser Glu Arg Val Gly Leu Ser Leu Glu Thr Ala
85     90     95
Asp Val Ser Leu Leu Gly Gln Ala Arg Lys Val Val Val Thr Lys Asp
100    105    110
Glu Thr Thr Ile Val Glu Gly Ser Gly Asp Ser Asp Ala Ile Ala Gly
115    120    125
Arg Val Ala Gln Ile Arg Ala Glu Ile Glu Asn Ser Asp Ser Asp Tyr
130    135    140
Asp Arg Glu Lys Leu Gln Glu Arg Leu Ala Lys Leu Ala Gly Gly Val
145    150    155    160
Ala Val Ile Lys Ala Gly Ala Ala Thr Glu Val Glu Leu Lys Glu Arg
165    170    175
Lys His Arg Ile Glu Asp Ala Val Arg Asn Ala Lys Ala Ala Val Glu
180    185    190
Glu Gly Ile Val Ala Gly Gly Gly Val Ala Leu Leu Gln Ser Ala Pro
195    200    205
Ala Leu Asp Asp Leu Gly Leu Thr Gly Asp Glu Ala Thr Gly Ala Asn

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210	215	220
Ile Val Arg Val Ala Leu Ser Ala Pro Leu Lys Gln Ile Ala Phe Asn		
225	230	235
Gly Gly Leu Glu Pro Gly Val Val Ala Glu Lys Val Ser Asn Leu Pro		240
	245	250
Ala Gly His Gly Leu Asn Ala Ala Thr Gly Glu Tyr Glu Asp Leu Leu		255
	260	265
Lys Ala Gly Val Ala Asp Pro Val Lys Val Thr Arg Ser Ala Leu Gln		270
	275	280
Asn Ala Ala Ser Ile Ala Ala Leu Phe Leu Thr Thr Glu Ala Val Val		285
	290	295
Ala Asp Lys Pro Glu Lys Ala Ser Ala Pro Ala Gly Asp Pro Thr Gly		300
305	310	315
Gly Met Gly Gly Met Asp Phe		320
	325	

<210> 163
 <211> 403
 <212> DNA
 <213> Mycobacterium vaccae

<400> 163

ggatccgcgg	caccggctgg	tgacgaccaa	gtacaaccgc	gcccgcacct	ggacggccga	60
gaactccgtc	ggcatcggcg	gcgcgtacct	gtgcatctac	gggatggagg	gccccggcgg	120
ctatcagttc	gtcggccgca	ccaccaggt	gtggagtcgt	taccgccaca	cggcgccggt	180
cgaaccggga	agtccctggc	tgtcgcggtt	tttcgaccga	atttcgtggt	atccggtgtc	240
ggccgaggag	ctgctggaat	tgcgagccga	catggccgca	ggccggggct	cggtcgacat	300
caccgacggc	gtgttctccc	tcgcgagca	cgaacgggtc	ctggccgaca	acgccgacga	360
catcgccgcg	ttcgttcccc	ggcaggcggc	cgcgttctcc	gcc		403

<210> 164
 <211> 336
 <212> DNA
 <213> Mycobacterium vaccae

<400> 164

cggaccgcgt	gggcgggcgc	cggcgagttc	gaccgcgccg	agaaagccgc	gtcgaaggcc	60
accgacgccg	ataccgggga	cctggtgctc	tacgacgggtg	cgagcgggtc	gacgctccgt	120
tcgcgtcgag	cgtgtggaag	gtcgacgtcg	ccgtcgggtga	ccgggtgggtg	gccggacagc	180
cgttgctggc	gctggaggcg	atgaagatgg	agaccgtgct	gcgcgccccg	gccgacgggg	240
tggtcaccca	gatactggtc	tcgctggggc	atctcgtcga	tcccggcacc	ccactgggtcg	300
tggtcggcac	cggagtgcgc	gcatgagcgc	cgtcga			336

<210> 165
 <211> 134
 <212> PRT
 <213> Mycobacterium vaccae

<400> 165

Asp Pro Arg His Arg Leu Val Thr Thr Lys Tyr Asn Pro Ala Arg Thr		
1	5	10
Trp Thr Ala Glu Asn Ser Val Gly Ile Gly Gly Ala Tyr Leu Cys Ile		15
	20	25
		30

Tyr Gly Met Glu Gly Pro Gly Gly Tyr Gln Phe Val Gly Arg Thr Thr
 35 40 45
 Gln Val Trp Ser Arg Tyr Arg His Thr Ala Pro Phe Glu Pro Gly Ser
 50 55 60
 Pro Trp Leu Leu Arg Phe Phe Asp Arg Ile Ser Trp Tyr Pro Val Ser
 65 70 75 80
 Ala Glu Glu Leu Leu Glu Leu Arg Ala Asp Met Ala Ala Gly Arg Gly
 85 90 95
 Ser Val Asp Ile Thr Asp Gly Val Phe Ser Leu Ala Glu His Glu Arg
 100 105 110
 Phe Leu Ala Asp Asn Ala Asp Asp Ile Ala Ala Phe Arg Ser Arg Gln
 115 120 125
 Ala Ala Ala Phe Ser Ala
 130

<210> 166
 <211> 108
 <212> PRT
 <213> Mycobacterium vaccae

<400> 166
 Arg Thr Ala Trp Ala Ala Ala Gly Glu Phe Asp Arg Ala Glu Lys Ala
 1 5 10 15
 Ala Ser Lys Ala Thr Asp Ala Asp Thr Gly Asp Leu Val Leu Tyr Asp
 20 25 30
 Gly Asp Glu Arg Val Asp Ala Pro Phe Ala Ser Ser Val Trp Lys Val
 35 40 45
 Asp Val Ala Val Gly Asp Arg Val Val Ala Gly Gln Pro Leu Leu Ala
 50 55 60
 Leu Glu Ala Met Lys Met Glu Thr Val Leu Arg Ala Pro Ala Asp Gly
 65 70 75 80
 Val Val Thr Gln Ile Leu Val Ser Ala Gly His Leu Val Asp Pro Gly
 85 90 95
 Thr Pro Leu Val Val Val Gly Thr Gly Val Arg Ala
 100 105

<210> 167
 <211> 31
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Made in a lab

<400> 167
 atagaattcg tccgacagtg ggacctcgag c

31

<210> 168
 <211> 27
 <212> DNA
 <213> Artificial Sequence
 <220>

<223> Made in a lab

<400> 168

atagaattcc caccgctca gccgccg

27

<210> 169

<211> 1111

<212> DNA

<213> Mycobacterium vaccae

<400> 169

gtccgacagt	gggacctcga	gcaccacgtc	acaggacagc	ggccccgcca	gcggcgccct	60
gcgcgtctcc	aactggccgc	tctatatggc	cgacggttct	atcgacagcg	tccagaccgc	120
ctcgggcatc	acggctcgact	acaaagaaga	cttcaacgac	aacgagcagt	ggttcgccaa	180
gggtcaaggag	ccgttgctgc	gcaagcagga	cataggcgcc	gacctggtga	tccccaccga	240
gttcatggcc	gcgcgcgtca	agggcctggg	atggctcaat	gagatcagcg	aagccggcgt	300
gccaatcgc	agaatctgc	gtcaggacct	gttggactcg	agcatcgacg	agggccgcaa	360
gttcaccgcg	ccgtacatga	ccggcatggg	cggtctcgcc	tacaacaagg	cagccaccgg	420
acgcgatatc	cgcaccatcg	acgacctctg	ggatcccgcg	ttcaagggcc	gcgtcagtct	480
gttctccgac	gtccaggacg	gcctcggcat	gatcatgctc	tcgcagggca	actcgccgga	540
gaatccgacc	accgagtcca	ttcagcaggc	ggtcgatctg	gtccgcgaac	agaacgacag	600
ggggtcagat	ccgtcgcttc	accggcaacg	actacgccga	cgacctggcc	gcagaaacat	660
cgccatcgcg	caggcggtact	ccgggtgacgt	cgtgcagctg	caggcgggaca	accccgatct	720
gcagttcatc	gttcccgaat	ccggcgggcga	ctggttcgtc	gacacgatgg	tgatcccgtg	780
caccacgcag	aaccagaagg	ccgcccaggc	gtggatcgac	tacatctacg	accgagccaa	840
ctacgccaa	ctggctcgct	tcacccagtt	cgtgcccgcg	ctctcggaca	tgaccgacga	900
actcgccaag	gtcgatcctg	catcggcgga	gaacccgctg	atcaaccctg	cggccgaggt	960
gcaggcgaac	ctgaagtctg	gggcggcact	gaccgacgag	cagacgcagg	agttcaacac	1020
tgcgtacgcc	gccgtcaccg	gcggctgacg	cggtggtagt	gccgatgcga	ggggcataaa	1080
tggccctgcg	gacgcgagga	gcataaatgg	c			1111

<210> 170

<211> 348

<212> PRT

<213> Mycobacterium vaccae

<400> 170

Ser	Asp	Ser	Gly	Thr	Ser	Ser	Thr	Thr	Ser	Gln	Asp	Ser	Gly	Pro	Ala
1				5				10					15		
Ser	Gly	Ala	Leu	Arg	Val	Ser	Asn	Trp	Pro	Leu	Tyr	Met	Ala	Asp	Gly
			20					25					30		
Phe	Ile	Ala	Ala	Phe	Gln	Thr	Ala	Ser	Gly	Ile	Thr	Val	Asp	Tyr	Lys
			35				40					45			
Glu	Asp	Phe	Asn	Asp	Asn	Glu	Gln	Trp	Phe	Ala	Lys	Val	Lys	Glu	Pro
			50			55					60				
Leu	Ser	Arg	Lys	Gln	Asp	Ile	Gly	Ala	Asp	Leu	Val	Ile	Pro	Thr	Glu
			65			70				75				80	
Phe	Met	Ala	Ala	Arg	Val	Lys	Gly	Leu	Gly	Trp	Leu	Asn	Glu	Ile	Ser
			85					90					95		
Glu	Ala	Gly	Val	Pro	Asn	Arg	Lys	Asn	Leu	Arg	Gln	Asp	Leu	Leu	Asp
			100					105					110		
Ser	Ser	Ile	Asp	Glu	Gly	Arg	Lys	Phe	Thr	Ala	Pro	Tyr	Met	Thr	Gly
			115				120						125		

Met Val Gly Leu Ala Tyr Asn Lys Ala Ala Thr Gly Arg Asp Ile Arg
 130 135 140
 Thr Ile Asp Asp Leu Trp Asp Pro Ala Phe Lys Gly Arg Val Ser Leu
 145 150 155 160
 Phe Ser Asp Val Gln Asp Gly Leu Gly Met Ile Met Leu Ser Gln Gly
 165 170 175
 Asn Ser Pro Glu Asn Pro Thr Thr Glu Ser Ile Gln Gln Ala Val Asp
 180 185 190
 Leu Val Arg Glu Gln Asn Asp Arg Gly Gln Ile Arg Arg Phe Thr Gly
 195 200 205
 Asn Asp Tyr Ala Asp Asp Leu Ala Ala Gly Asn Ile Ala Ile Ala Gln
 210 215 220
 Ala Tyr Ser Gly Asp Val Val Gln Leu Gln Ala Asp Asn Pro Asp Leu
 225 230 235 240
 Gln Phe Ile Val Pro Glu Ser Gly Gly Asp Trp Phe Val Asp Thr Met
 245 250 255
 Val Ile Pro Tyr Thr Thr Gln Asn Gln Lys Ala Ala Glu Ala Trp Ile
 260 265 270
 Asp Tyr Ile Tyr Asp Arg Ala Asn Tyr Ala Lys Leu Val Ala Phe Thr
 275 280 285
 Gln Phe Val Pro Ala Leu Ser Asp Met Thr Asp Glu Leu Ala Lys Val
 290 295 300
 Asp Pro Ala Ser Ala Glu Asn Pro Leu Ile Asn Pro Ser Ala Glu Val
 305 310 315 320
 Gln Ala Asn Leu Lys Ser Trp Ala Ala Leu Thr Asp Glu Gln Thr Gln
 325 330 335
 Glu Phe Asn Thr Ala Tyr Ala Ala Val Thr Gly Gly
 340 345

<210> 171

<211> 1420

<212> DNA

<213> Mycobacterium vaccae

<220>

<221> unsure

<222> (955)...(955)

<221> unsure

<222> (973)...(973)

<400> 171

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gctggccccgc	ccgggtgcaac	tcctgcgtac	ctacatcctg	ccgctgggcg	cgttgctgct	180
cctgctggta	caggcgatgg	agatctccga	cgacgccacg	tcggtaacgt	tggtcgccac	240
cctgttcggc	gtcgtgttgt	tgacgttggt	gctgtccggg	ctcaacgcca	ccctcatcca	300
gggcgcacca	gaagacagct	ggcgcaggcg	gattccgtcg	atcttcctcg	acgtcgcgcg	360
cttcgcgctg	atcgcggtcg	gtatcacctg	gatcatggcc	tatgtctggg	gcgcgaacgt	420
ggggggcctg	ttcacgcac	tgggcgtcac	ttccatcggt	cttggcctgg	ctctgcagaa	480
ttcggtcggt	cagatcatct	cgggtctgct	gctgctgttc	gagcaaccgt	tccggctcgg	540
cgactggatc	accgtcccca	ccgcggcggg	ccggccgtcc	gcccacggcc	gcgtggtgga	600
agtcaactgg	cgtgcaacac	atatcgacac	cggcggaac	ctgctggtaa	tgcccaacgc	660

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cgaactcgcc ggcgcgtcgt tcaccaatta cagccggccc gtgggagagc accggctgac 720
cgctcgtcacc accttcaacg ccgcggacac ccccgatgat gtctgcgaga tgctgtcgtc 780
ggtcgcggcg tcgctgcccg aactgcgcac cgacggacag atcgccacgc tctatctcgg 840
tgccggccgaa tacgagaagt cgatcccgtt gcacacaccc gcggtggacg actcggtcag 900
gagcacgtac ctgcatggg tctggtacgc cgcgcgccgg caggaacttc gcctnaacgg 960
cgtcgccgac ganttcgaca cgccggaacg gatcgctcg gccatgcggg ctgtggcgtc 1020
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ttacggcaac ggggaacgcc tccagcagcc gggtcaggta ccgaccggga tgaggttcat 1140
cgtagacggc aggggtgagtc tgtccgtgat cgatcaggac ggcgacgtga tcccggcgcg 1200
gggtgctcgag cgtggcgact tcctggggca gaccacgtg acgcgggaac cgggtactggc 1260
gaccgcgcac gcgctggagg aagtcaccgt gctggagatg gcccgtagcg agatcgagcg 1320
cctggtgcac cgaaagccga tcctgctgca cgtgatcggg gccgtgatcg ccgaccggcg 1380
cgcgcacgaa cttcggttga tggcggactc gcaggactga 1420

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<210> 172

<211> 471

<212> PRT

<213> Mycobacterium vaccae

<220>

<221> UNSURE

<222> (318)...(318)

<221> UNSURE

<222> (324)...(324)

<400> 172

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Met Ser Ser Val Leu Asn Ser Thr Trp Leu Ala Trp Ala Val Ala Val
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Ala Val Gly Phe Pro Val Leu Leu Val Val Leu Thr Glu Val His Asn
          20          25          30
Ala Leu Arg Arg Arg Gly Ser Ala Leu Ala Arg Pro Val Gln Leu Leu
          35          40          45
Arg Thr Tyr Ile Leu Pro Leu Gly Ala Leu Leu Leu Leu Leu Val Gln
          50          55          60
Ala Met Glu Ile Ser Asp Asp Ala Thr Ser Val Arg Leu Val Ala Thr
65          70          75          80
Leu Phe Gly Val Val Leu Leu Thr Leu Val Leu Ser Gly Leu Asn Ala
          85          90          95
Thr Leu Ile Gln Gly Ala Pro Glu Asp Ser Trp Arg Arg Arg Ile Pro
          100          105          110
Ser Ile Phe Leu Asp Val Ala Arg Phe Ala Leu Ile Ala Val Gly Ile
          115          120          125
Thr Val Ile Met Ala Tyr Val Trp Gly Ala Asn Val Gly Gly Leu Phe
          130          135          140
Thr Ala Leu Gly Val Thr Ser Ile Val Leu Gly Leu Ala Leu Gln Asn
145          150          155          160
Ser Val Gly Gln Ile Ile Ser Gly Leu Leu Leu Leu Phe Glu Gln Pro
          165          170          175
Phe Arg Leu Gly Asp Trp Ile Thr Val Pro Thr Ala Ala Gly Arg Pro
          180          185          190
Ser Ala His Gly Arg Val Val Glu Val Asn Trp Arg Ala Thr His Ile
          195          200          205

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Asp Thr Gly Gly Asn Leu Leu Val Met Pro Asn Ala Glu Leu Ala Gly
 210 215 220
 Ala Ser Phe Thr Asn Tyr Ser Arg Pro Val Gly Glu His Arg Leu Thr
 225 230 235 240
 Val Val Thr Thr Phe Asn Ala Ala Asp Thr Pro Asp Asp Val Cys Glu
 245 250 255
 Met Leu Ser Ser Val Ala Ala Ser Leu Pro Glu Leu Arg Thr Asp Gly
 260 265 270
 Gln Ile Ala Thr Leu Tyr Leu Gly Ala Ala Glu Tyr Glu Lys Ser Ile
 275 280 285
 Pro Leu His Thr Pro Ala Val Asp Asp Ser Val Arg Ser Thr Tyr Leu
 290 295 300
 Arg Trp Val Trp Tyr Ala Ala Arg Arg Gln Glu Leu Arg Xaa Asn Gly
 305 310 315 320
 Val Ala Asp Xaa Phe Asp Thr Pro Glu Arg Ile Ala Ser Ala Met Arg
 325 330 335
 Ala Val Ala Ser Thr Leu Arg Leu Ala Asp Asp Glu Gln Gln Glu Ile
 340 345 350
 Ala Asp Val Val Arg Leu Val Arg Tyr Gly Asn Gly Glu Arg Leu Gln
 355 360 365
 Gln Pro Gly Gln Val Pro Thr Gly Met Arg Phe Ile Val Asp Gly Arg
 370 375 380
 Val Ser Leu Ser Val Ile Asp Gln Asp Gly Asp Val Ile Pro Ala Arg
 385 390 395 400
 Val Leu Glu Arg Gly Asp Phe Leu Gly Gln Thr Thr Leu Thr Arg Glu
 405 410 415
 Pro Val Leu Ala Thr Ala His Ala Leu Glu Glu Val Thr Val Leu Glu
 420 425 430
 Met Ala Arg Asp Glu Ile Glu Arg Leu Val His Arg Lys Pro Ile Leu
 435 440 445
 Leu His Val Ile Gly Ala Val Ile Ala Asp Arg Arg Ala His Glu Leu
 450 455 460
 Arg Leu Met Asp Ser Gln Asp
 465 470

<210> 173

<211> 2172

<212> DNA

<213> Mycobacterium vaccae

<400> 173

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gggcgctacc	acctcctgtc	gcggatgagc	atccagtgcca	agttgctgct	gatgctgctt	120
ctgaccagca	ttctctcggc	tgcggtgggc	ggtttcatcg	gctatcagtc	cggacgggtcc	180
tcgctgcgcg	categgtgtt	cgaccgcctc	accgacatcc	gcgagtcgca	gtcgcgcggg	240
ttggagaatc	agttcgcgga	cctgaagaac	tcgatgggtga	tttactcgcg	cggcagcact	300
gccacggagg	cgatcggcgc	gttcagcgac	ggtttccgct	agctcggcga	tgcgacgatac	360
aataccgggc	aggcggcgct	attgcgcgct	tactacgacc	ggacgttcgc	caacaccacc	420
ctcgacgaca	gcggaaaccg	cgtcgacgtc	cgcgcgctca	tcccgaatc	caacccccag	480
cgctatctgc	aggcgctcta	taccccgccg	tttcagaact	gggagaaggc	gatcgcgcttc	540
gacgacgcgc	gcgacggcag	cgccctggctg	gccgccaatg	ccagattcaa	cgagttcttc	600
cgcgagatcg	tgccacgctt	caacttcgag	gatctgatgc	tgctcgacct	cgagggcaac	660
gtggtgtact	ccgcctacaa	ggggccggat	ctcggggacaa	acatcgctcaa	cggcccctat	720

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cgcaaccggg aactgtcgga agcctacgag aaggcggtcg cgtcgaaactc gatcgactat 780
gtcgggtgtca ccgacttcgg gtggtacctg cctgccgagg aaccgaccgc ctgggttcctg 840
tccccgggtcg gggtgaagga ccgagtcgac ggtgtgatgg cgggtccagtt cccgatcgcg 900
cggatcaacg aattgatgac ggcgcgggga cagtggcggtg acaccgggat gggagacacc 960
ggtagagacca tcctggtcgg accggacaat ctgatgcgct cggactcccg gctgttcgcg 1020
gagaaccggg agaagttcct ggccgacgtc gtcgaggggg gaaccccgcc ggaggtcgcc 1080
gacgaatcgg ttgaccgccc cggcaccacg ctggtgcagc cggtgaccac ccgctccgtc 1140
gaggaggccc aacgcggcaa caccgggacg acgatcgagg acgactatct cggccacgag 1200
gcgttacagg cgtactcacc ggtggacctg cggggactgc actgggtgat cgtggccaag 1260
atcgacaccg acgaggcggt cgccccgggtg gcgcagttca ccaggaccct ggtgctgtcg 1320
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cgtccgatcc ggcggttgca ggccggcgcc cagcagatca gcggcggtga ctaccgcctc 1440
gctctgccgg tgtgtctctg tgacgaattc ggcgatctga caacagcttt caacgacatg 1500
agtcgcaatc tgtcgatcaa ggacgagctg ctccggcgagg agcgcgccga gaaccaacgg 1560
ctgatgctgt cctgatgcc cgaaccgggtg atgcagcgct acctcgaccg ggaggagacg 1620
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ctggccagct gcgggttagg cgtgccgcgg ctggacaacg tccggcgcac ggtcaatttc 1860
gcgatcgaaa tggaccgcat catcgaccgg cagccgcgcc agtccgggca cgacctgcgg 1920
ctccgcgcgg gcatcgacac cgggtcggcg gccagcgggc tgggtggggcg gtccacgttg 1980
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cagcccgcca tctacgtcac ctccgggtg cagcaggtca tgcaggaaac tctcgacttc 2100
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caccggcgat ga 2172

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<210> 174

<211> 722

<212> PRT

<213> Mycobacterium vaccae

<400> 174

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Met Thr Ile Leu Pro Trp Asn Ala Arg Thr Ser Glu His Pro Thr Arg
1          5          10          15
Lys Arg Arg Gly Arg Tyr His Leu Leu Ser Arg Met Ser Ile Gln Ser
20          25          30
Lys Leu Leu Leu Met Leu Leu Leu Thr Ser Ile Leu Ser Ala Ala Val
35          40          45
Val Gly Phe Ile Gly Tyr Gln Ser Gly Arg Ser Ser Leu Arg Ala Ser
50          55          60
Val Phe Asp Arg Leu Thr Asp Ile Arg Glu Ser Gln Ser Arg Gly Leu
65          70          75          80
Glu Asn Gln Phe Ala Asp Leu Lys Asn Ser Met Val Ile Tyr Ser Arg
85          90          95
Gly Ser Thr Ala Thr Glu Ala Ile Gly Ala Phe Ser Asp Gly Phe Arg
100          105          110
Gln Leu Gly Asp Ala Thr Ile Asn Thr Gly Gln Ala Ala Ser Leu Arg
115          120          125
Arg Tyr Tyr Asp Arg Thr Phe Ala Asn Thr Thr Leu Asp Asp Ser Gly
130          135          140
Asn Arg Val Asp Val Arg Ala Leu Ile Pro Lys Ser Asn Pro Gln Arg
145          150          155          160
Tyr Leu Gln Ala Leu Tyr Thr Pro Pro Phe Gln Asn Trp Glu Lys Ala

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1000

Arg Thr Leu His Asp Gly Tyr Leu Ala Ser Cys Gly Leu Gly Val Pro
 595 600 605
 Arg Leu Asp Asn Val Arg Arg Thr Val Asn Phe Ala Ile Glu Met Asp
 610 615 620
 Arg Ile Ile Asp Arg His Ala Ala Glu Ser Gly His Asp Leu Arg Leu
 625 630 635 640
 Arg Ala Gly Ile Asp Thr Gly Ser Ala Ala Ser Gly Leu Val Gly Arg
 645 650 655
 Ser Thr Leu Ala Tyr Asp Met Trp Gly Ser Ala Val Asp Val Ala Asn
 660 665 670
 Gln Val Gln Arg Gly Ser Pro Gln Pro Gly Ile Tyr Val Thr Ser Arg
 675 680 685
 Val His Glu Val Met Gln Glu Thr Leu Asp Phe Val Ala Ala Gly Glu
 690 695 700
 Val Val Gly Glu Arg Gly Val Glu Thr Val Trp Arg Leu Gln Gly His
 705 710 715 720
 Arg Arg

<210> 175
 <211> 898
 <212> DNA
 <213> Mycobacterium vaccae

<400> 175
 gagcaaccgt tccggctcgg cgactggatc accgtcccca ccgcggcggg ccggccgtcc 60
 gccacaggcc gcgtggtgga agtcaactgg cgtgcaacac atatcgacac cggcggcaac 120
 ctgctggttaa tgcccaacgc cgaactcgcc ggcgcgctcgt tcaccaatta cagccggccc 180
 gtgggagagc accggctgac cgtcgtcacc accttcaacg ccgcggacac ccccgatgat 240
 gtctgcgaga tgcgtcgtc ggtcgcggcg tcgctgcccg aactgcgcac cgacggacag 300
 atcgccacgc tctatctcgg tcgggccgaa tacgagaagt cgatcccgtt gcacacaccc 360
 gcggtggacg actcggctcag gagcacgtac ctgcgatggg tctggtacgc cgcgcgccgg 420
 caggaaacttc gcctaacggc gtcgccgacg attcgacacg ccggaacgga tcgcctcggc 480
 catgcgggct gtggcgtcca cactgcgctt ggcagacgac gaacagcagg agatcgccga 540
 cgtggtgcgt ctggtccgtt acggcaacgg ggaacgcctc cagcagccgg gtcaggtagc 600
 gaccgggatg aggttcacg tagacggcag ggtgagtcgt tccgtgatcg atcaggacgg 660
 cgacgtgatc ccggcgccgg tgctcgagcg tggcgacttc ctggggcaga ccacgtgac 720
 gcgggaaccg gtactggcga ccgcgcacgc gctggaggaa gtcaccgtgc tggagatggc 780
 ccgtgacgag atcgagcgcc tgggtgcacc aaagccgatc ctgctgcacg tgatcggggc 840
 cgtgatcgcc gaccggcgcg cgcacgaact tcggttgatg gcggactcgc aggactga 898

<210> 176
 <211> 2013
 <212> DNA
 <213> Mycobacterium vaccae

<400> 176
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 cgcgagtcgc agtcgcgcgg gttggagaat cagttcgcgg acctgaagaa ctcgatggtg 120
 atttactcgc gcggcagcac tgccacggag gcgatcggcg cgttcagcga cggtttcgt 180
 cagctcggcg atgcgacgat caataccggg caggcggcgt cattgcgcg ttactacgac 240
 cggacgttcg ccaacaccac cctcgacgac agcggaaacc gcgtcgacgt ccgcgcgtc 300
 atccccgaat ccaaccccca gcgctatctg caggcgctct ataccccgcc gtttcagaac 360

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tgggagaagg cgatcgcgtt cgacgacgcg cgcgacggca ggccttggtc ggccgccaat 420
gccagattca acgagttctt ccgcgagatc gtgcaccgct tcaacttcga ggatctgatg 480
ctgctcgacc tcgagggcaa cgtggtgtac tccgcctaca aggggcccga tctcgggaca 540
aacatcgtca acggccccta tcgcaaccgg gaactgtcgg aagcctacga gaaggcggtc 600
gcgtcgaact cgatcgacta tgtcgggtgc accgacttcg ggtggtacct gcctgccgag 660
gaaccgaccg cctggttctt gtccccggtc gggttgaagg accgagtcga cgggtgtgatg 720
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cactgggtga tcgtggccaa gatcgacacc gacgaggcgt tcgccccggg ggcgcagttc 1140
accaggaccg tgggtgtgtc gacggtgatc atcatcttcg gcgtgtcgt ggccggccatg 1200
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agcggcggtg actacgcct cgctctgccg gtgttgtctc gtgacgaatt cggcgatctg 1320
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gacatgatgg gcctcgacga gttgtcgcgc atgttgacct ccgaggaact gatggtggtg 1560
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cggacgctgc acgacgggta cctggccagc tgcgggtag gcgtgccgcg gctggacaac 1680
gtccggcgca cggtaattt cgcgatcgaa atggaccgca tcatcgaccg gcacgccgcc 1740
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ctggtggggc ggtccacgtt ggcgtacgac atgtggggtt cggcggtcga tgtcgctaac 1860
caggtgcagc gcggctcccc ccagccggc atctacgtca cctcgcgggt gcacgaggtc 1920
atgcaggaaa ctctcgactt cgtcgccgcc ggggaggtcg tcggcgagcg cggcgtcgag 1980
acggtctggc ggttcgaggg ccaccggcga tga 2013

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<210> 177

<211> 297

<212> PRT

<213> Mycobacterium vaccae

<220>

<221> UNSURE

<222> (145)...(145)

<221> UNSURE

<222> (151)...(151)

<400> 177

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Glu Gln Pro Phe Arg Leu Gly Asp Trp Ile Thr Val Pro Thr Ala Ala
 1             5             10             15
Gly Arg Pro Ser Ala His Gly Arg Val Val Glu Val Asn Trp Arg Ala
          20          25          30
Thr His Ile Asp Thr Gly Gly Asn Leu Leu Val Met Pro Asn Ala Glu
      35          40          45
Leu Ala Gly Ala Ser Phe Thr Asn Tyr Ser Arg Pro Val Gly Glu His
      50          55          60
Arg Leu Thr Val Val Thr Phe Asn Ala Ala Asp Thr Pro Asp Asp
      65          70          75          80
Val Cys Glu Met Leu Ser Ser Val Ala Ala Ser Leu Pro Glu Leu Arg

```

85						90						95					
Thr	Asp	Gly	Gln	Ile	Ala	Thr	Leu	Tyr	Leu	Gly	Ala	Ala	Glu	Tyr	Glu		
100						105						110					
Lys	Ser	Ile	Pro	Leu	His	Thr	Pro	Ala	Val	Asp	Asp	Ser	Val	Arg	Ser		
115						120						125					
Thr	Tyr	Leu	Arg	Trp	Val	Trp	Tyr	Ala	Ala	Arg	Arg	Gln	Glu	Leu	Arg		
130						135						140					
Xaa	Asn	Gly	Val	Ala	Asp	Xaa	Phe	Asp	Thr	Pro	Glu	Arg	Ile	Ala	Ser		
145						150						155					
Ala	Met	Arg	Ala	Val	Ala	Ser	Thr	Leu	Arg	Leu	Ala	Asp	Asp	Glu	Gln		
165						170						175					
Gln	Glu	Ile	Ala	Asp	Val	Val	Arg	Leu	Val	Arg	Tyr	Gly	Asn	Gly	Glu		
180						185						190					
Arg	Leu	Gln	Gln	Pro	Gly	Gln	Val	Pro	Thr	Gly	Met	Arg	Phe	Ile	Val		
195						200						205					
Asp	Gly	Arg	Val	Ser	Leu	Ser	Val	Ile	Asp	Gln	Asp	Gly	Asp	Val	Ile		
210						215						220					
Pro	Ala	Arg	Val	Leu	Glu	Arg	Gly	Asp	Phe	Leu	Gly	Gln	Thr	Thr	Leu		
225						230						235					
Thr	Arg	Glu	Pro	Val	Leu	Ala	Thr	Ala	His	Ala	Leu	Glu	Glu	Val	Thr		
245						250						255					
Val	Leu	Glu	Met	Ala	Arg	Asp	Glu	Ile	Glu	Arg	Leu	Val	His	Arg	Lys		
260						265						270					
Pro	Ile	Leu	Leu	His	Val	Ile	Gly	Ala	Val	Ala	Asp	Arg	Arg	Ala	His		
275						280						285					
Glu	Leu	Arg	Leu	Met	Asp	Ser	Gln	Asp									
290						295											

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<210> 178
<211> 670
<212> PRT
<213> Mycobacterium vaccae
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<400> 178															
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Leu	Thr	Asp	Ile	Arg	Glu	Ser	Gln	Ser	Arg	Gly	Leu	Glu	Asn	Gln	Phe
			20			25						30			
Ala	Asp	Leu	Lys	Asn	Ser	Met	Val	Ile	Tyr	Ser	Arg	Gly	Ser	Thr	Ala
			35			40						45			
Thr	Glu	Ala	Ile	Gly	Ala	Phe	Ser	Asp	Gly	Phe	Arg	Gln	Leu	Gly	Asp
			50			55						60			
Ala	Thr	Ile	Asn	Thr	Gly	Gln	Ala	Ala	Ser	Leu	Arg	Arg	Tyr	Tyr	Asp
65				70			75						80		
Arg	Thr	Phe	Ala	Asn	Thr	Thr	Leu	Asp	Asp	Ser	Gly	Asn	Arg	Val	Asp
			85			90						95			
Val	Arg	Ala	Leu	Ile	Pro	Lys	Ser	Asn	Pro	Gln	Arg	Tyr	Leu	Gln	Ala
			100			105						110			
Leu	Tyr	Thr	Pro	Pro	Phe	Gln	Asn	Trp	Glu	Lys	Ala	Ile	Ala	Phe	Asp
			115			120						125			
Asp	Ala	Arg	Asp	Gly	Ser	Ala	Trp	Ser	Ala	Ala	Asn	Ala	Arg	Phe	Asn
			130			135						140			
Glu	Phe	Phe	Arg	Glu	Ile	Val	His	Arg	Phe	Asn	Phe	Glu	Asp	Leu	Met

145		150		155		160
Leu	Leu	Asp	Leu	Glu	Gly	Pro
		165		170		175
Asp	Leu	Gly	Thr	Asn	Ile	Val
		180		185		190
Ser	Glu	Ala	Tyr	Glu	Lys	Ala
		195		200		205
Gly	Val	Thr	Asp	Phe	Gly	Trp
		210		215		220
Trp	Phe	Leu	Ser	Pro	Val	Gly
225				230		235
Ala	Val	Gln	Phe	Pro	Ile	Ala
		245		250		255
Gly	Gln	Trp	Arg	Asp	Thr	Gly
		260		265		270
Val	Gly	Pro	Asp	Asn	Leu	Met
		275		280		285
Asn	Arg	Glu	Lys	Phe	Leu	Ala
		290		295		300
Glu	Val	Ala	Asp	Glu	Ser	Val
305				310		315
Pro	Val	Thr	Thr	Arg	Ser	Val
		325		330		335
Thr	Thr	Ile	Glu	Asp	Asp	Tyr
		340		345		350
Ser	Pro	Val	Asp	Leu	Pro	Gly
		355		360		365
Asp	Thr	Asp	Glu	Ala	Phe	Ala
		370		375		380
Val	Leu	Ser	Thr	Val	Ile	Ile
385				390		395
Leu	Leu	Ala	Arg	Leu	Phe	Val
		405		410		415
Ala	Gln	Gln	Ile	Ser	Gly	Gly
		420		425		430
Ser	Arg	Asp	Glu	Phe	Gly	Asp
		435		440		445
Arg	Asn	Leu	Ser	Ile	Lys	Asp
		450		455		460
Asn	Gln	Arg	Leu	Met	Leu	Ser
465				470		475
Tyr	Leu	Asp	Gly	Glu	Glu	Thr
		485		490		495
Val	Ile	Phe	Ala	Asp	Met	Met
		500		505		510
Thr	Ser	Glu	Leu	Met	Val	Val
		515		520		525
Asp	Ala	Ala	Ala	Glu	Ser	Leu
		530		535		540
Asp	Gly	Tyr	Leu	Ala	Ser	Cys
545				550		555
Val	Arg	Arg	Thr	Val	Asn	Phe
		565		570		575

Arg His Ala Ala Glu Ser Gly His Asp Leu Arg Leu Arg Ala Gly Ile
 580 585 590
 Asp Thr Gly Ser Ala Ala Ser Gly Leu Val Gly Arg Ser Thr Leu Ala
 595 600 605
 Tyr Asp Met Trp Gly Ser Ala Val Asp Val Ala Asn Gln Val Gln Arg
 610 615 620
 Gly Ser Pro Gln Pro Gly Ile Tyr Val Thr Ser Arg Val His Glu Val
 625 630 635 640
 Met Gln Glu Thr Leu Asp Phe Val Ala Ala Gly Glu Val Val Gly Glu
 645 650 655
 Arg Gly Val Glu Thr Val Trp Arg Leu Gln Gly His Arg Arg
 660 665 670

<210> 179
 <211> 520
 <212> DNA
 <213> Mycobacterium vaccae

<400> 179
 gtgatcgacg aaaccctctt ccatgccgag gagaagatgg agaaggccgt ctcggtggca 60
 ccgacgacc tggcgctgat tcgtaccggc cgcgcgaacc ccggcatgtt caaccggatc 120
 aacatcgact actacggcgc ctccaccccg atcacgcagc tgtccagcat caacgtgccc 180
 gaggcgcgca tgggtggtgat caagccctac gaggcgagcc agctgcgcct catcgaggat 240
 gcgatccgca actccgacct cggcgctcaat ccgaccaacg acggcaacat catccgggtg 300
 tcgatcccgcc agctcaccca ggagcgccgc cgcgacctgg tcaagcaggc caaggccaag 360
 ggcgaggacg ccaaggtgtc ggtgcgcaac atccgtcgca acgatatgaa cacctttcgc 420
 atcgacccgg tacggctgcc gacgccaccg ccgtcgtaga agcgacagag gatcgcaggt 480
 aacggtattg gccacgcctt ctgtggcggg ccgacaccac 520

<210> 180
 <211> 1071
 <212> DNA
 <213> Mycobacterium vaccae

<400> 180
 cgtggggaag gattgcactc tatgagcgaa atcgcccgtc cctggcggggt tctggcaggt 60
 ggcacggtg cctgcgcgcg gggatatgcc ggggtgctga gcacgcgggt caccacggcg 120
 tcggcccagc cgggcctccc gcagcccccg ctgcccggcc ctgccacagt gacgcaaacc 180
 gtcacggttg cgcccaacgc cgcgccacaa ctcatcccgc gcccgggtgt gacgcctgcc 240
 accggcggcg ccgcgcgggt gcccgcgggg gtgagcgccc cggcggtcgc gccggccccc 300
 gcgctgcccg cccgcccgggt gtccacgacg gcccgcggcca cctcgggcac gctcagcgag 360
 ttcttcgccc ccaagggcgt cacgatggag ccgcagtcca gccgcgactt ccgcgccttc 420
 aacatcgctg tgccgaagcc gcggggctgg gagcacatcc cggaccgaa cgtgccggac 480
 gcgttcgccc tgctggccga cggggtcggc ggcaacggcc tgtactcgtc gaacgcccag 540
 gtggtggtct acaaactcgt cggcgagttc gaccccaagg aagcgatcag ccacggcttc 600
 gtcgacagcc agaagctgcc ggcgtggcgt tccaccgacg cgtcgctggc cgacttcggc 660
 ggaatgccgt cctcgctgat cgagggcacc taccgcgaga acaacatgaa gctgaacacg 720
 tcccggcgcc acgtcattgc caccgcgggg cccgaccact acctggtgtc gctgtcggtg 780
 accaccagcg tcgaacaggc cgtggccgaa gccgcggagg ccaccgacgc gattgtcaac 840
 ggcttcaagg tcagcgtttc ggggtccgggt ccggccgcac cgccacctgc acccggtgcc 900
 cccgggtgcc cggcgccccc cggcgccccc gcgctgcgcg tggcgcgtgc accacccccg 960
 gtccecgctg ttcccgcgtt ggcgccccgc ccacagctgc tgggactgca gggatagacg 1020
 tcgtcgcccc ccgggcgaag cctggcgccc gggggacgac ggccccttct t 1071

<210> 181
 <211> 152
 <212> PRT
 <213> Mycobacterium vaccae

<400> 181
 Val Ile Asp Glu Thr Leu Phe His Ala Glu Glu Lys Met Glu Lys Ala
 1 5 10 15
 Val Ser Val Ala Pro Asp Asp Leu Ala Ser Ile Arg Thr Gly Arg Ala
 20 25 30
 Asn Pro Gly Met Phe Asn Arg Ile Asn Ile Asp Tyr Tyr Gly Ala Ser
 35 40 45
 Thr Pro Ile Thr Gln Leu Ser Ser Ile Asn Val Pro Glu Ala Arg Met
 50 55 60
 Val Val Ile Lys Pro Tyr Glu Ala Ser Gln Leu Arg Leu Ile Glu Asp
 65 70 75 80
 Ala Ile Arg Asn Ser Asp Leu Gly Val Asn Pro Thr Asn Asp Gly Asn
 85 90 95
 Ile Ile Arg Val Ser Ile Pro Gln Leu Thr Glu Glu Arg Arg Arg Asp
 100 105 110
 Leu Val Lys Gln Ala Lys Ala Lys Gly Glu Asp Ala Lys Val Ser Val
 115 120 125
 Arg Asn Ile Arg Arg Asn Asp Met Asn Thr Phe Arg Ile Ala Pro Val
 130 135 140
 Arg Leu Pro Thr Pro Pro Ser
 145 150

<210> 182
 <211> 331
 <212> PRT
 <213> Mycobacterium vaccae

<400> 182
 Met Ser Glu Ile Ala Arg Pro Trp Arg Val Leu Ala Gly Gly Ile Gly
 1 5 10 15
 Ala Cys Ala Ala Gly Ile Ala Gly Val Leu Ser Ile Ala Val Thr Thr
 20 25 30
 Ala Ser Ala Gln Pro Gly Leu Pro Gln Pro Pro Leu Pro Ala Pro Ala
 35 40 45
 Thr Val Thr Gln Thr Val Thr Val Ala Pro Asn Ala Ala Pro Gln Leu
 50 55 60
 Ile Pro Arg Pro Gly Val Thr Pro Ala Thr Gly Gly Ala Ala Ala Val
 65 70 75 80
 Pro Ala Gly Val Ser Ala Pro Ala Val Ala Pro Ala Pro Ala Leu Pro
 85 90 95
 Ala Arg Pro Val Ser Thr Ile Ala Pro Ala Thr Ser Gly Thr Leu Ser
 100 105 110
 Glu Phe Phe Ala Ala Lys Gly Val Thr Met Glu Pro Gln Ser Ser Arg
 115 120 125
 Asp Phe Arg Ala Leu Asn Ile Val Leu Pro Lys Pro Arg Gly Trp Glu
 130 135 140
 His Ile Pro Asp Pro Asn Val Pro Asp Ala Phe Ala Val Leu Ala Asp

```

145          150          155          160
Arg Val Gly Gly Asn Gly Leu Tyr Ser Ser Asn Ala Gln Val Val Val
          165          170          175
Tyr Lys Leu Val Gly Glu Phe Asp Pro Lys Glu Ala Ile Ser His Gly
          180          185          190
Phe Val Asp Ser Gln Lys Leu Pro Ala Trp Arg Ser Thr Asp Ala Ser
          195          200          205
Leu Ala Asp Phe Gly Gly Met Pro Ser Ser Leu Ile Glu Gly Thr Tyr
          210          215          220
Arg Glu Asn Asn Met Lys Leu Asn Thr Ser Arg Arg His Val Ile Ala
225          230          235          240
Thr Ala Gly Pro Asp His Tyr Leu Val Ser Leu Ser Val Thr Thr Ser
          245          250          255
Val Glu Gln Ala Val Ala Glu Ala Ala Glu Ala Thr Asp Ala Ile Val
          260          265          270
Asn Gly Phe Lys Val Ser Val Pro Gly Pro Gly Pro Ala Ala Pro Pro
          275          280          285
Pro Ala Pro Gly Ala Pro Gly Val Pro Pro Ala Pro Gly Ala Pro Ala
          290          295          300
Leu Pro Leu Ala Val Ala Pro Pro Pro Ala Pro Ala Val Pro Ala Val
305          310          315          320
Ala Pro Ala Pro Gln Leu Leu Gly Leu Gln Gly
          325          330

```

<210> 183
 <211> 207
 <212> DNA
 <213> Mycobacterium vaccae

```

<400> 183
acctacgagt tcgagaacaa ggtcacgggc ggccgcatcc cgcgcgagta catcccgtcg      60
gtggatgccg gcgcgcagga cgccatgcag tacggcgtgc tggccggcta cccgctggtt      120
aacgtcaagc tgacgtgtgt cgacggtgcc taccacgaag tcgactcgtc ggaaatggca      180
ttcaagggtg cgggtccca ggtcata                                     207

```

<210> 184
 <211> 69
 <212> PRT
 <213> Mycobacterium vaccae

```

<400> 184
Thr Tyr Glu Phe Glu Asn Lys Val Thr Gly Gly Arg Ile Pro Arg Glu
1          5          10          15
Tyr Ile Pro Ser Val Asp Ala Gly Ala Gln Asp Ala Met Gln Tyr Gly
          20          25          30
Val Leu Ala Gly Tyr Pro Leu Val Asn Val Lys Leu Thr Leu Leu Asp
          35          40          45
Gly Ala Tyr His Glu Val Asp Ser Ser Glu Met Ala Phe Lys Val Ala
          50          55          60
Gly Ser Gln Val Ile
65

```

<210> 185

<211> 898
 <212> DNA
 <213> Mycobacterium vaccae

<220>
 <221> unsure
 <222> (637) ... (637)

<221> unsure
 <222> (662) ... (662)

<400> 185

cgacctccac	ccgggctgta	ggccaaccac	taggctggtc	accagtagtc	gacggcacac	60
ttcaccgaaa	aaatgaggac	agaggagaca	cccgtgacga	tccgtggttg	tgtgaacggc	120
ttcggccgta	tcggacgcaa	cttcttccgc	gcgctggacg	cgcagaaggc	cgaaggcaag	180
aacaaggaca	tcgagatcgt	cgcgggtcaac	gacctcaccg	acaacgccac	gctggcgcac	240
ctgctgaagt	tcgactcgat	cctggggccgg	ctgccctacg	acgtgagcct	cgaaggcgag	300
gacaccatcg	tcgtcggcag	caccaagatc	aaggcgctcg	aggtcaagga	aggcccggcg	360
gcgctgccct	ggggcgacct	ggggcgctgac	gtcgtcgtcg	agtcacccgg	catcttcacc	420
aagcgcgaca	aggcccaggg	ccacctcgac	gcgggcgcca	agaagggtcat	catctccgcg	480
ccggccaccg	atgaggacat	caccatcgtg	ctcggcgctca	acgacgacaa	gtacgacggc	540
agccagaaca	tcattctccaa	cgcgtcgtgc	accacgaact	gcctcggccc	gctggcggaag	600
gtcatcaacg	acgagttcgg	catcgtcaag	ggcctgntga	ccaccatcca	cgcctacacc	660
cnggtccaga	acctgcagga	cggcccgcac	aaggatctgc	gccgggcccc	cgccgcccgcg	720
ctgaacatcg	tgccgacctc	caccggtgcc	gccaaggcca	tcggactggg	gctgcccgcag	780
ctgaagggca	agctcgacgg	ctacgcgctg	cgggtgccga	tccccaccgg	ctcggtcacc	840
gacctgaccg	ccgagctggg	caagtcggcc	accgtggacg	agatcaacgc	cgcgatga	898

<210> 186
 <211> 268
 <212> PRT
 <213> Mycobacterium vaccae

<220>
 <221> UNSURE
 <222> (182) ... (182)

<221> UNSURE
 <222> (190) ... (190)

<400> 186

Val	Thr	Ile	Arg	Val	Gly	Val	Asn	Gly	Phe	Gly	Arg	Ile	Gly	Arg	Asn
1				5					10					15	
Phe	Phe	Arg	Ala	Leu	Asp	Ala	Gln	Lys	Ala	Glu	Gly	Lys	Asn	Lys	Asp
			20					25					30		
Ile	Glu	Ile	Val	Ala	Val	Asn	Asp	Leu	Thr	Asp	Asn	Ala	Thr	Leu	Ala
			35				40					45			
His	Leu	Leu	Lys	Phe	Asp	Ser	Ile	Leu	Gly	Arg	Leu	Pro	Tyr	Asp	Val
50					55						60				
Ser	Leu	Glu	Gly	Glu	Asp	Thr	Ile	Val	Val	Gly	Ser	Thr	Lys	Ile	Lys
65				70					75					80	
Ala	Leu	Glu	Val	Lys	Glu	Gly	Pro	Ala	Ala	Leu	Pro	Trp	Gly	Asp	Leu
			85					90						95	

Gly Val Asp Val Val Val Glu Ser Thr Gly Ile Phe Thr Lys Arg Asp
 100 105 110
 Lys Ala Gln Gly His Leu Asp Ala Gly Ala Lys Lys Val Ile Ile Ser
 115 120 125
 Ala Pro Ala Thr Asp Glu Asp Ile Thr Ile Val Leu Gly Val Asn Asp
 130 135 140
 Asp Lys Tyr Asp Gly Ser Gln Asn Ile Ile Ser Asn Ala Ser Cys Thr
 145 150 155 160
 Thr Asn Cys Leu Gly Pro Leu Ala Lys Val Ile Asn Asp Glu Phe Gly
 165 170 175
 Ile Val Lys Gly Leu Xaa Thr Thr Ile His Ala Tyr Thr Xaa Val Gln
 180 185 190
 Asn Leu Gln Asp Gly Pro His Lys Asp Leu Arg Arg Ala Arg Ala Ala
 195 200 205
 Ala Leu Asn Ile Val Pro Thr Ser Thr Gly Ala Ala Lys Ala Ile Gly
 210 215 220
 Leu Val Leu Pro Glu Leu Lys Gly Lys Leu Asp Gly Tyr Ala Leu Arg
 225 230 235 240
 Val Pro Ile Pro Thr Gly Ser Val Thr Asp Leu Thr Ala Glu Leu Gly
 245 250 255
 Lys Ser Ala Thr Val Asp Glu Ile Asn Ala Ala Met
 260 265

<210> 187
 <211> 41
 <212> PRT
 <213> Mycobacterium vaccae

<220>
 <221> UNSURE
 <222> (39)...(39)

<400> 187
 Met Asn Lys Ala Glu Leu Ile Asp Val Leu Thr Glu Lys Leu Gly Ser
 1 5 10 15
 Asp Arg Arg Gln Ala Thr Ala Ala Val Glu Asn Val Val Asp Thr Ile
 20 25 30
 Val Ala Ala Val Pro Lys Xaa Val Val
 35 40

<210> 188
 <211> 26
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Made in a lab

<221> unsure
 <222> (12)...(12)

<400> 188
 atgaayaarg cngarctsat ygaygt

<210> 189
 <211> 20
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Made in a lab

<400> 189
 atsgtrtgva cvacgttytc 20

<210> 190
 <211> 84
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Made in a lab

<221> unsure
 <222> (2)...(2)

<400> 190
 gnactcattg acgtactcac tgagaagctg ggctcggatt gtcggcaagc gactgcggca 60
 atggagaacg tgggccacac cata 84

<210> 191
 <211> 337
 <212> DNA
 <213> Mycobacterium vaccae

<220>
 <221> unsure
 <222> (2)...(2)

<400> 191
 gnactcattg acgtactcac tgagaagctg ggctcggatt gtcggcaagc gactgcggcg 60
 gtggagaatg ttgtcgacac catcgtgcgc gccgtgcaca aggggtgagag cgtcaccatc 120
 acgggcttcg gtgttttcga gcagcgtcgt cgcgcagcac gcgtggcacg caatccgcgc 180
 accggcgaga ccgtgaaggt caagcccacc tcagtcccggt cattccgtcc cggcgctcag 240
 ttcaaggctg ttgtctctgg cgcacagaag cttccggccg aggggtccggc ggtcaagcgc 300
 ggtgtgaccg cgacgagcac cgcccgaag gcagcca 337

<210> 192
 <211> 111
 <212> PRT
 <213> Mycobacterium vaccae

<220>
 <221> UNSURE
 <222> (1)...(1)

<400> 192

```

Xaa Leu Ile Asp Val Leu Thr Glu Lys Leu Gly Ser Asp Arg Gln Ala
 1           5           10           15
Thr Ala Ala Val Glu Asn Val Val Asp Thr Ile Val Arg Ala Val His
          20           25           30
Lys Gly Glu Ser Val Thr Ile Thr Gly Phe Gly Val Phe Glu Gln Arg
          35           40           45
Arg Arg Ala Ala Arg Val Ala Arg Asn Pro Arg Thr Gly Glu Thr Val
          50           55           60
Lys Val Lys Pro Thr Ser Val Pro Ala Phe Arg Pro Gly Ala Gln Phe
65           70           75           80
Lys Ala Val Val Ser Gly Ala Gln Lys Leu Pro Ala Glu Gly Pro Ala
          85           90           95
Val Lys Arg Gly Val Thr Ala Thr Ser Thr Ala Arg Lys Ala Ala
          100           105           110

```

<210> 193

<211> 1164

<212> DNA

<213> Mycobacterium vaccae

<400> 193

```

ggtggcgcg atcgagaagc gcccgccccg gttcacgggc gcctgatcat ggtgcgggcg      60
gcgctgcgct acggcttcgg gacggcctca ctgctggccg gcgggttcgt gctgcgcgcc      120
ctgcagggca cgctgccgc cctcggcgcg actccgggag aggtcgcgcc ggtggcgcg      180
cgctcgccga actaccgca cggcaagttc gtcaacctgg agccccgtc gggcatcacg      240
atggatcgcg acctgcagcg gatgctgttg cgcgatctgg ccaacgccgc atcccagggc      300
aagccgcccc gaccgatccc gctggccgag ccgccgaagg gggatcccac tcccgcgcgc      360
gogggggcca gctggtacgg ccattccagc gtgctgatcg aggtcgacgg ctaccgcgtg      420
ctggccgacc cgggtgtggag caacagatgt tcgccctcac gggcggtcgg accgcagcgc      480
atgcacgacg tcccgtgccc gctggaggcg cttcccgccg tggacgcggg ggtgatcagc      540
cacgaccact acgaccacct cgacatcgac accatcgtcg cgttggcgca caccagcgg      600
gccccgttcg tgggtgccgtt gggcatcggc gcacacctgc gcaagtgggg cgtccccgag      660
gcgcggatcg tcgagttgga ctggcacgaa gccaccgca tagacgacct gacgctggtc      720
tgcacccccg cccggcactt ctccggacgg ttgttctccc gcgactcgac gctgtgggcg      780
tcgtgggtgg tcaccggctc gtcgcacaag gcgttcttcg gtggcgacac cggatacacg      840
aagagcttcg ccgagatcgg cgacgagtac ggtccgttcg atctgaccct gctgccgatc      900
ggggcctacc atcccgcgtt cggcgacatc cacatgaacc ccgaggaggc ggtgcgcgcc      960
catctggacc tgaccgaggt ggacaacagc ctgatgggtg ccatccactg ggcgacattc     1020
cgcctcgccc cgcacccgtg gtcgagccc gccgaacgcc tgctgaccgc tgccgacgcc     1080
gagcgggtac gcctgaccgt gccgattccc ggtcagcggg tggacccgga gtcgacgttc     1140
gaccctgggt ggcggttctg aacc                                     1164

```

<210> 194

<211> 370

<212> PRT

<213> Mycobacterium vaccae

<400> 194

```

Met Val Arg Ala Ala Leu Arg Tyr Gly Phe Gly Thr Ala Ser Leu Leu
 1           5           10           15
Ala Gly Gly Phe Val Leu Arg Ala Leu Gln Gly Thr Pro Ala Ala Leu
          20           25           30

```

Gly Ala Thr Pro Gly Glu Val Ala Pro Val Ala Arg Arg Ser Pro Asn
 35 40 45
 Tyr Arg Asp Gly Lys Phe Val Asn Leu Glu Pro Pro Ser Gly Ile Thr
 50 55 60
 Met Asp Arg Asp Leu Gln Arg Met Leu Leu Arg Asp Leu Ala Asn Ala
 65 70 75 80
 Ala Ser Gln Gly Lys Pro Pro Gly Pro Ile Pro Leu Ala Glu Pro Pro
 85 90 95
 Lys Gly Asp Pro Thr Pro Ala Pro Ala Ala Ala Ser Trp Tyr Gly His
 100 105 110
 Ser Ser Val Leu Ile Glu Val Asp Gly Tyr Arg Val Leu Ala Asp Pro
 115 120 125
 Val Trp Ser Asn Arg Cys Ser Pro Ser Arg Ala Val Gly Pro Gln Arg
 130 135 140
 Met His Asp Val Pro Val Pro Leu Glu Ala Leu Pro Ala Val Asp Ala
 145 150 155 160
 Val Val Ile Ser His Asp His Tyr Asp His Leu Asp Ile Asp Thr Ile
 165 170 175
 Val Ala Leu Ala His Thr Gln Arg Ala Pro Phe Val Val Pro Leu Gly
 180 185 190
 Ile Gly Ala His Leu Arg Lys Trp Gly Val Pro Glu Ala Arg Ile Val
 195 200 205
 Glu Leu Asp Trp His Glu Ala His Arg Ile Asp Asp Leu Thr Leu Val
 210 215 220
 Cys Thr Pro Ala Arg His Phe Ser Gly Arg Leu Phe Ser Arg Asp Ser
 225 230 235 240
 Thr Leu Trp Ala Ser Trp Val Val Thr Gly Ser Ser His Lys Ala Phe
 245 250 255
 Phe Gly Gly Asp Thr Gly Tyr Thr Lys Ser Phe Ala Glu Ile Gly Asp
 260 265 270
 Glu Tyr Gly Pro Phe Asp Leu Thr Leu Leu Pro Ile Gly Ala Tyr His
 275 280 285
 Pro Ala Phe Ala Asp Ile His Met Asn Pro Glu Glu Ala Val Arg Ala
 290 295 300
 His Leu Asp Leu Thr Glu Val Asp Asn Ser Leu Met Val Pro Ile His
 305 310 315 320
 Trp Ala Thr Phe Arg Leu Ala Pro His Pro Trp Ser Glu Pro Ala Glu
 325 330 335
 Arg Leu Leu Thr Ala Ala Asp Ala Glu Arg Val Arg Leu Thr Val Pro
 340 345 350
 Ile Pro Gly Gln Arg Val Asp Pro Glu Ser Thr Phe Asp Pro Trp Trp
 355 360 365
 Arg Phe
 370

<210> 195

<211> 650

<212> DNA

<213> Mycobacterium vaccae

<400> 195

gacacaccag caccactgtt aacctcgcta gatcagtcgg ccgaacggaa ggacagccgt
 gaccctgaaa accctagtca ccagcatgac cgctggggca gcagcagccg caacactcgg

60
 120

```

cgctgccgcc gtgggtgtga cctcgattgc cgtcggtgcg ggtgtcgccg gcgcgtcgcc 180
cgcggtgctg aacgcaccgc tgctttccgc cctgcccc gatctgcagg gaccgctggt 240
ctccaccttg agcgcgctgt cgggcccggg ctcttcgccc ggcgccaagg ccacctacgt 300
ccagggcggt ctcgcccgca tcgaggcccg ggtggccgac agcggataca gcaacgccgc 360
ggccaagggc tacttcccgc tgagcttcac cgtcgccggc atcgaccaga acggtccgat 420
cgtgaccgcc aacgtcaccg cggcgggccc gacgggccc gtggccaccc agccgctgac 480
gttcacgcgc gggccgagcc cgaccggatg gcagctgtcc aagcagtcgc cactggccct 540
gatgtccgcg gtgggtgata tcccgacga ttctggtccg cagcgccgtc acatgtgtgg 600
cggcgctcgg gctgggtggg tgcctgggcg gctgcgcgca agatgaacat 650

```

<210> 196

<211> 159

<212> PRT

<213> Mycobacterium vaccae

<400> 196

```

Met Thr Ala Gly Ala Ala Ala Ala Thr Leu Gly Ala Ala Ala Val
1      5      10      15
Gly Val Thr Ser Ile Ala Val Gly Ala Gly Val Ala Gly Ala Ser Pro
20     25     30
Ala Val Leu Asn Ala Pro Leu Leu Ser Ala Pro Ala Pro Asp Leu Gln
35     40     45
Gly Pro Leu Val Ser Thr Leu Ser Ala Leu Ser Gly Pro Gly Ser Phe
50     55     60
Ala Gly Ala Lys Ala Thr Tyr Val Gln Gly Gly Leu Gly Arg Ile Glu
65     70     75     80
Ala Arg Val Ala Asp Ser Gly Tyr Ser Asn Ala Ala Ala Lys Gly Tyr
85     90     95
Phe Pro Leu Ser Phe Thr Val Ala Gly Ile Asp Gln Asn Gly Pro Ile
100    105    110
Val Thr Ala Asn Val Thr Ala Ala Pro Thr Gly Ala Val Ala Thr
115    120    125
Gln Pro Leu Thr Phe Ile Ala Gly Pro Ser Pro Thr Gly Trp Gln Leu
130    135    140
Ser Lys Gln Ser Ala Leu Ala Leu Met Ser Ala Val Ile Ala Ala
145    150    155

```

<210> 197

<211> 285

<212> PRT

<213> Mycobacterium vaccae

<400> 197

```

Met Gln Val Arg Arg Val Leu Gly Ser Val Gly Ala Ala Val Ala Val
1      5      10      15
Ser Ala Ala Leu Trp Gln Thr Gly Val Ser Ile Pro Thr Ala Ser Ala
20     25     30
Asp Pro Cys Pro Asp Ile Glu Val Ile Phe Ala Arg Gly Thr Gly Ala
35     40     45
Glu Pro Gly Leu Gly Trp Val Gly Asp Ala Phe Val Asn Ala Leu Arg
50     55     60
Pro Lys Val Gly Glu Gln Ser Val Gly Thr Tyr Ala Val Asn Tyr Pro
65     70     75     80

```


Ala Gly Phe Asp Phe Asp Lys Ser Ala Pro Met Gly Ala Ala Asp Ala
 85 90 95
 Ser Gly Arg Val Gln Trp Met Ala Asp Asn Cys Pro Asp Thr Lys Leu
 100 105 110
 Val Leu Gly Gly Met Ser Gln Gly Ala Gly Val Ile Asp Leu Ile Thr
 115 120 125
 Val Asp Pro Arg Pro Leu Gly Arg Phe Thr Pro Thr Pro Met Pro Pro
 130 135 140
 Arg Val Ala Asp His Val Ala Ala Val Val Val Phe Gly Asn Pro Leu
 145 150 155 160
 Arg Asp Ile Arg Gly Gly Gly Pro Leu Pro Gln Met Ser Gly Thr Tyr
 165 170 175
 Gly Pro Lys Ser Ile Asp Leu Cys Ala Leu Asp Asp Pro Phe Cys Ser
 180 185 190
 Pro Gly Phe Asn Leu Pro Ala His Phe Ala Tyr Ala Asp Asn Gly Met
 195 200 205
 Val Glu Glu Ala Ala Asn Phe Ala Arg Leu Glu Pro Gly Gln Ser Val
 210 215 220
 Glu Leu Pro Glu Ala Pro Tyr Leu His Leu Phe Val Pro Arg Gly Glu
 225 230 235 240
 Val Thr Leu Glu Asp Ala Gly Pro Leu Arg Glu Gly Asp Ala Val Arg
 245 250 255
 Phe Thr Ala Ser Gly Gly Gln Arg Val Thr Ala Thr Ala Pro Ala Glu
 260 265 270
 Ile Leu Val Trp Glu Met His Ala Gly Leu Gly Ala Ala
 275 280 285

<210> 198

<211> 743

<212> DNA

<213> Mycobacterium vaccae

<400> 198

ggatccgcgg	caccggctgg	tgacgaccaa	gtacaacccg	gcccgcacct	ggacggccga	60
gaactccgtc	ggcatcggcg	gcgcgtacct	gtgcatctac	gggatggagg	gccccggcgg	120
ctatcagttc	gtcggccgca	ccaccaggt	gtggagtcgt	taccgccaca	cggcgccgtt	180
cgaaccggga	agtccctggc	tgtgctggtt	tttcgaccga	atttcgtggt	atccggtgtc	240
ggccgaggag	ctgctggaat	tgcgagccga	catggccgca	ggccgggggt	cggtcgacat	300
caccgacggc	gtgtttctcc	tcgccgagca	cgaacggttc	ctggccgaca	acgccgacga	360
catcgccgcg	ttccgttccc	ggcaggcggc	cgcgttctcc	gccgagcgga	ccgcgtgggc	420
ggccgcccgc	gagttcgacc	gcgccgagaa	agccgcgtcg	aaggccaccg	acgccgatac	480
cggggacctg	gtgctctacg	acggtgacga	gcgggtcgac	gctccgttcg	cgtcgagcgt	540
gtggaagggtc	gacgtcgccg	tcggtgaccg	gggtggtggc	ggacagccgt	tgctggcgct	600
ggaggcgatg	aagatggaga	ccgtgctgcg	cgccccggcc	gacgggggtg	tcaccagat	660
cctggtctcc	gctgggcatc	tcgtcgatcc	cggcacccca	ctggtcgtgg	tcggcaccgg	720
agtgcgcgca	tgagcgccgt	cga				743

<210> 199

<211> 243

<212> PRT

<213> Mycobacterium vaccae

<400> 199
 Asp Pro Arg His Arg Leu Val Thr Thr Lys Tyr Asn Pro Ala Arg Thr
 1 5 10 15
 Trp Thr Ala Glu Asn Ser Val Gly Ile Gly Gly Ala Tyr Leu Cys Ile
 20 25 30
 Tyr Gly Met Glu Gly Pro Gly Gly Tyr Gln Phe Val Gly Arg Thr Thr
 35 40 45
 Gln Val Trp Ser Arg Tyr Arg His Thr Ala Pro Phe Glu Pro Gly Ser
 50 55 60
 Pro Trp Leu Leu Arg Phe Phe Asp Arg Ile Ser Trp Tyr Pro Val Ser
 65 70 75 80
 Ala Glu Glu Leu Leu Glu Leu Arg Ala Asp Met Ala Ala Gly Arg Gly
 85 90 95
 Ser Val Asp Ile Thr Asp Gly Val Phe Ser Leu Ala Glu His Glu Arg
 100 105 110
 Phe Leu Ala Asp Asn Ala Asp Asp Ile Ala Ala Phe Arg Ser Arg Gln
 115 120 125
 Ala Ala Ala Phe Ser Ala Glu Arg Thr Ala Trp Ala Ala Ala Gly Glu
 130 135 140
 Phe Asp Arg Ala Glu Lys Ala Ala Ser Lys Ala Thr Asp Ala Asp Thr
 145 150 155 160
 Gly Asp Leu Val Leu Tyr Asp Gly Asp Glu Arg Val Asp Ala Pro Phe
 165 170 175
 Ala Ser Ser Val Trp Lys Val Asp Val Ala Val Gly Asp Arg Val Val
 180 185 190
 Ala Gly Gln Pro Leu Leu Ala Leu Glu Ala Met Lys Met Glu Thr Val
 195 200 205
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/31, C07K 14/35, C12N 15/62, C07K 19/00, 16/12, A61K 39/04, 48/00, G01N 33/68	A3	(11) International Publication Number: WO 99/32634 (43) International Publication Date: 1 July 1999 (01.07.99)																	
(21) International Application Number: PCT/NZ98/00189 (22) International Filing Date: 23 December 1998 (23.12.98) (30) Priority Data: <table border="0"><tr><td>08/997,362</td><td>23 December 1997 (23.12.97)</td><td>US</td></tr><tr><td>08/997,080</td><td>23 December 1997 (23.12.97)</td><td>US</td></tr><tr><td>08/996,624</td><td>23 December 1997 (23.12.97)</td><td>US</td></tr><tr><td>09/095,855</td><td>11 June 1998 (11.06.98)</td><td>US</td></tr><tr><td>09/156,181</td><td>17 September 1998 (17.09.98)</td><td>US</td></tr><tr><td>09/205,426</td><td>4 December 1998 (04.12.98)</td><td>US</td></tr></table> (71) Applicant (for all designated States except US): GENESIS RE-SEARCH & DEVELOPMENT CORPORATION LIMITED [NZ/NZ]; 1 Fox Street, Parnell, Auckland (NZ). (72) Inventors; and (75) Inventors/Applicants (for US only): TAN, Paul [NZ/NZ]; 26B Alberon Street, Parnell, Auckland (NZ). WATSON, James [NZ/NZ]; 769 Riddell Road, Auckland (NZ). VISSER, Elizabeth, S. [ZA/NZ]; 3 Lynbrooke Avenue, Blockhouse Bay, Auckland (NZ). SKINNER, Margot, A. [NZ/NZ]; 113 West End Road, Westmere, Auckland (NZ). PRESTIDGE, Ross, L. [NZ/NZ]; 20 Hepburn Street, Freemans Bay, Auckland (NZ).	08/997,362	23 December 1997 (23.12.97)	US	08/997,080	23 December 1997 (23.12.97)	US	08/996,624	23 December 1997 (23.12.97)	US	09/095,855	11 June 1998 (11.06.98)	US	09/156,181	17 September 1998 (17.09.98)	US	09/205,426	4 December 1998 (04.12.98)	US	(74) Agents: BENNETT, Michael, Roy et al.; Russell McVeagh West-Walker, The Todd Building, Level 5, 171-177 Lambton Quay, Wellington 6001 (NZ). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> (88) Date of publication of the international search report: 2 December 1999 (02.12.99)
08/997,362	23 December 1997 (23.12.97)	US																	
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09/205,426	4 December 1998 (04.12.98)	US																	
(54) Title: COMPOSITIONS DERIVED FROM <i>MYCOBACTERIUM VACCAE</i> AND METHODS FOR THEIR USE (57) Abstract <p>The present invention provides compositions which are present in or may be derived from <i>Mycobacterium vaccae</i>, together with methods for their use in the treatment, prevention and detection of disorders including infectious diseases, immune disorders and cancer. Methods for enhancing the immune response to an antigen including administration of <i>M. vaccae</i> culture filtrate, delipidated <i>M. vaccae</i> cells, delipidated and deglycolipidated <i>M. vaccae</i> cells depleted of mycolic acids, and delipidated and deglycolipidated <i>M. vaccae</i> cells depleted of mycolic acids and arabinogalactan are also provided.</p>																			

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INTERNATIONAL SEARCH REPORT

International Application No

F Z 98/00189

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/31 C07K14/35 C12N15/62 C07K19/00 C07K16/12
 A61K39/04 A61K48/00 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X, L	WO 98 08542 A (GENESIS RESEARCH & DEV CORP L1) 5 March 1998 see the whole document L: Priority	1-42
X A	EP 0 763 361 A (UNIV LONDON) 19 March 1997 see the whole document	24,25 1-23, 26-43
X A	WO 91 02542 A (UNIV LONDON) 7 March 1991 see the whole document, especially page 6, lines 9-18	24,25 1-23, 26-43
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Further documents are listed in the continuation of box C.



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Date of the actual completion of the international search

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Date of mailing of the international search report

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Mandl, B

INTERNATIONAL SEARCH REPORT

International Application No
/NZ 98/00189

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SKINNER M. A. ET AL.: "IMMUNIZATION WITH HEAT-KILLED MYCOBACTERIUM VACCAE STIMULATES CD8+CYTOTOXIC T CELLS SPECIFIC FOR MACROPHAGES INFECTED WITH MYCOBACTERIUM TUBERCULOSIS" INFECTION AND IMMUNITY, vol. 65, no. 11, 1 November 1997, pages 4525-4530, XP002060474	24,25
A	see the whole document	1-23, 26-43
X	--- STANFORD J. L. ET AL.: "Mycobacterium vaccae in immunoprophylaxis and immunotherapy of leprosy and tuberculosis." VACCINE, vol. 8, December 1990, pages 525-530, XP002106918	24,25
A	see the whole document	1-23, 26-43
A	--- STANFORD J L: "IMPROVING ON BCG" APMIS, vol. 99, no. 2, 1 January 1991, pages 103-113, XP000616012	16,43
A	--- KAPUR V. ET AL.: "RAPID MYCOBACTERIUM SPECIES ASSIGNMENT AND UNAMBIGUOUS IDENTIFICATION OF MUTATIONS ASSOCIATED WITH ANTIMICROBIALS RESISTANCE IN MYCOBACTERIUM TUBERCULOSIS BY AUTOMATED DNA SEQUENCING" ARCHIVES OF PATHOLOGY & LABORATORY MEDICINE, vol. 119, no. 2, 1 February 1995, pages 131-138, XP000572767	1-43
	see the whole document & EMBL database entry MV17958; accession number U17958; 22-Dec-1994; Kapur V. et al.: 'Mycobacterium vaccae 65 kDa heat shock protein gene, partial cds.' see abstract	
A	--- SOINI H. AND VILJANEN M. K.: "Diversity of the 32-kilodalton protein gene may form a basis for species determination of potentially pathogenic mycobacterial species." JOURNAL OF CLINICAL MICROBIOLOGY, vol. 35, no. 3, March 1997, pages 769-773, XP002094599	1-43
	see figure 1 --- -/-	

INTERNATIONAL SEARCH REPORT

International Application No

98/00189

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>YOUNG R. A. ET AL.: "DISSECTION OF MYCOBACTERIUM TUBERCULOSIS ANTIGENS USING RECOMBINANT DNA"</p> <p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,</p> <p>vol. 82, 1 May 1985, pages 2583-2587,</p> <p>XP002034045</p> <p>see the whole document</p> <p>-----</p>	1-43

INTERNATIONAL SEARCH REPORT

International application No.

PCT/NZ 98/ 00189

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Please see FURTHER INFORMATION Sheet PCT/ISA/210

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

REMARK: Although claims 17-26 and 43 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition. And although claims 27 and 28 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

/NZ 98/00189

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